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ANALYTICAL EDITION

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Application of Infrared Spectroscopy to Industrial Research

NORMAN WRIGHT, The Dow Chemical Company, Midland, Mich.

THE range of the infrared spectrum discussed in this paper extends from about 2.5μ (25,000 Å.) to approximately 15μ. Since photographic plates are sensitive to wave lengths no longer than 1.3μ, the spectrum here mentioned is detected and measured through the heating effect of the radiation, in the present case by a thermopile and galvanometer.

It has long been recognized, through such early work as that of Coblentz (*2*) in 1905 on the infrared spectra of a large number of compounds, that the selective absorption (or emission) of infrared radiation arises in the mutual vibrations of the atoms constituting the molecules. A molecule does not absorb radiation of all wave lengths but selects only a few narrow wave-length intervals which are known as absorption bands. The resulting absorption pattern is characteristic of the molecule.

Theoretical treatment of the vibrations of molecules and the correlation with infrared spectra has been summarized for the simpler molecules by Dennison (*3*). He pointed out that the vibration frequencies within a molecule are determined by the masses of the atoms, the strength of the forces which bind them, and the geometrical structure of the molecule. In the case of organic compounds there is only slight dependence on the state of aggregation of the molecules, and the factors mentioned lead to vibration frequencies corresponding to wave lengths lying for the greater part in the spectral range 2.5 to 15μ.

Inorganic compounds do not present as favorable a field for infrared spectroscopy as do the organic. Chief of the disadvantages encountered with inorganic compounds is the fact that water, the commonest solvent, is nearly opaque to infrared waves longer than 1.5μ. A second disadvantage is the great width of absorption (or reflection) bands of inorganic compounds. The field of organic chemistry, on the other hand, lends itself particularly well to the methods of infrared spectroscopy, and applications in this field only are discussed in the present paper.

In addition to the general fact that the infrared spectrum of an organic molecule is characteristic of that molecule, it is well known that certain groupings or subgroups of atoms within molecules behave more or less independently of the rest of the molecule and give rise to characteristic absorption bands. For example, the O—H group gives rise to a band in the vicinity of 2.75μ (in unassociated molecules), irrespective of the type of molecule containing this hydroxyl group. Compounds with the C≡N group possess a band at about 4.45μ, and those with the C=O group have a band in the interval 5.6 to 5.9μ. These cases and similar ones have long been known; considerable literature of such subgroup bands or "linkage" bands has been built up in recent years (*1, 8, 12*).

A great many applications of infrared spectroscopy can be made in the field of industrial organic chemistry, both as a tool for research and for actual control of plant processes. A list of such applications would include: (1) identification of organic compounds, (2) detection and identification of small amounts of impurities in organic compounds, (3) accurate quantitative determination of such impurities, (4) study of reaction mechanisms and speeds, and detection of intermediates, (5) study of isomerism and tautomerism, (6) study of association and compound formation, (7) study of polymerization and copolymerization in the field of plastics, (8) determination of geometrical structures, moments of inertia, and bond lengths, (9) determination of force constants and dissociation constants, (10) calculation of specific heats and other thermodynamic constants, and (11) study of crystal structure through use of polarized radiation. There are no doubt other applications.

In view of the work of Coblentz (*2*) and others before 1905, pointing directly to many of the above applications, the question arises why these have not long ago been put into industrial practice. The answer lies almost entirely in the severe experimental difficulties encountered by early workers. From the standpoint of optics there was no great obstacle, but the extremely high sensitivity of the radiation-detecting devices to all sorts of external disturbances has been overcome only in recent years. This explains the scarcity of reports on efforts to utilize infrared spectroscopy in industry, particularly in the analytical field.

Two instances of analysis by means of infrared which are no doubt adaptable to industrial use include a method developed by McAlister (*9*) for the accurate and rapid analysis of carbon dioxide in air, and an ingenious method devised by Pfund (*11*) for the determination of simple gases such as carbon dioxide, carbon monoxide, and methane without the use of a spectrometer. There are no reports, however, of the industrial use of infrared spectroscopy for quantitative analysis of more complex organic compounds. It is therefore the purpose of this paper to describe in some detail the methods of qualitative and quantitative analysis by means of infrared spectroscopy which are being employed on a routine basis in an industrial laboratory.

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Apparatus

The spectrograph is an automatically recording instrument employing a 60° prism of rock salt in a Littrow mounting. It was designed to meet the requirements of producing and rapidly recording an infrared spectrum of the proper quality and yet retaining the simplicity and dependability vital to industrial application. In some respects it resembles the spectrograph of Strong and Randall (*13*) which, however, uses the more complicated Wadsworth-Littrow mounting. The instrument was built to order by the shop of the Physics Department of the University of Michigan. A diagram of the optical system is shown in Figure 1.

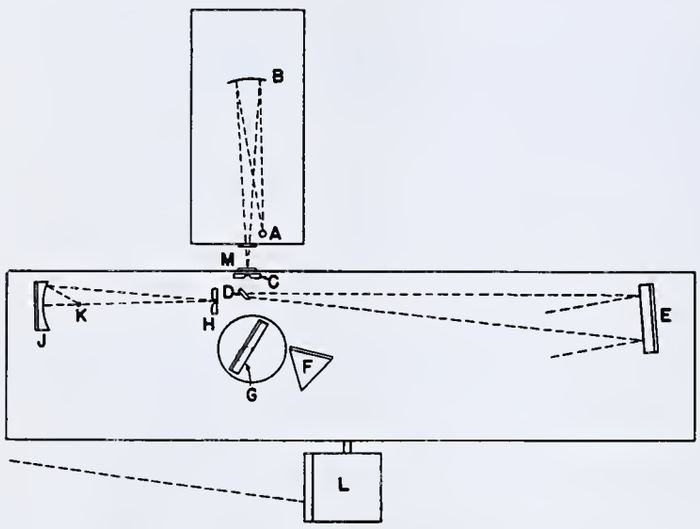

FIGURE 1. DIAGRAM OF INFRARED SPECTROGRAPH

The source of the infrared radiation is a globar element, *A* (12.5 cm., 5 inches long), mounted in a water-cooled jacket. The radiation is focused by the concave mirror, *B*, onto the slit of the spectrograph, *C*. All mirrors of the instrument are aluminized on the front surface. Space is left at *M* for inserting into the radiation path the cell containing the sample whose absorption spectrum is to be recorded. After passing through the sample and through the slit of the spectrograph the diverging radiation falls on the collimating mirror, *E*. This mirror is an on-axis paraboloid of 15-cm. (6-inch) diameter and a focal length of 90 cm. (36 inches). The collimated radiation passes through the prism, *F*, which has faces 10 × 8 cm. and refracting angle about 60°. After passing through the prism the radiation is returned by the plane (Littrow) mirror, *G*, again passing through the prism and again falling on mirror *E*. Mirror *E* then focuses the radiation into a spectrum, a narrow wave-length interval of which is selected by the second slit, *H*. From the slit the radiation is strongly condensed on the receiver of the thermopile, *K*, by the ellipsoidal mirror, *J*, which has focal distances of 7.5 and 37.5 cm. (3 and 15 inches). The thermopile is of the Pfund (*10*) design and has two junctions; only one of these receives the radiation, the other serving as a compensator.

Connected to this is a galvanometer of moderately high sensitivity (L. & N. Type HS, 1.5-second period), the deflections of which are amplified 10 to 20 times by means of a very simple dry-plate photocell relay. The final deflection is produced in a galvanometer of rugged construction (L. & N. Type R). Each galvanometer is mounted on a type of Julius vibrationless support. It is, of course, possible to achieve deflections of suitable magnitude with a single, very sensitive galvanometer, but such galvanometers usually have periods of from 7 to 10 seconds which would lead to excessive recording times. Use of the amplifier yields a high sensitivity without sacrifice of time, and permits employment of more stable galvanometers.

Automatic scanning of the spectrum is accomplished by slowly rotating *G* by a motor drive, causing the spectrum to move slowly over the selector slit, *H*. Simultaneously the deflection produced in the final galvanometer is photographically recorded on the synchronized drum camera, *L*.

A photograph of the instrument and room is shown in Figure 2. The radiation source and focusing mirror are enclosed in an air-tight case, as is also the spectrometer proper. This allows removal of water vapor from the radiation path by drying agents placed within the cases. The source-box can be readily moved toward or away from the spectrometer to permit insertion of sample cells of different length. The room is lined with sheet metal to shield against the electromagnetic effects of the high-potential spark sources used in the spectrographic laboratory nearby. This instrument has been in constant service for nearly 3 years.

Several records of infrared spectra made with this instrument are shown in Figures 3, 5, and 6. These records (15 cm., 6 inches, wide by 50 cm., 20 inches, long) are graphs of galvanometer deflection produced by the transmitted radiation *vs.* wave length as indicated in microns along the bottom edge. Zero deflection of the galvanometer is at the bottom edge of the records and is marked by the dots at the beginning and end of each section of the recording. The vertical fiducial lines are photographed on the records at intervals of 20 units of a revolution counter geared to the Littrow mirror drive. Infrared wave lengths are easily read from a calibration curve obtained with the use of known standard wave lengths.

The manner in which a record is made is illustrated in Figure 3 by the spectrum obtained with no material other than air in the radiation path.

A record is made by starting at the long wave-length end and recording toward shorter wave lengths. The radiation, of course, grows more intense following approximately the black body radiation law, approaching a maximum at about 1.5μ. When the deflection grows too large for the camera, the instrument is stopped and the two slits are narrowed; this decreases the amount of radiation falling on the thermopile and at the same time increases the resolution of the spectrometer. This process is repeated five times during the recording of a spectrum from 14 to 2μ. The time required for the complete recording is 25 minutes.

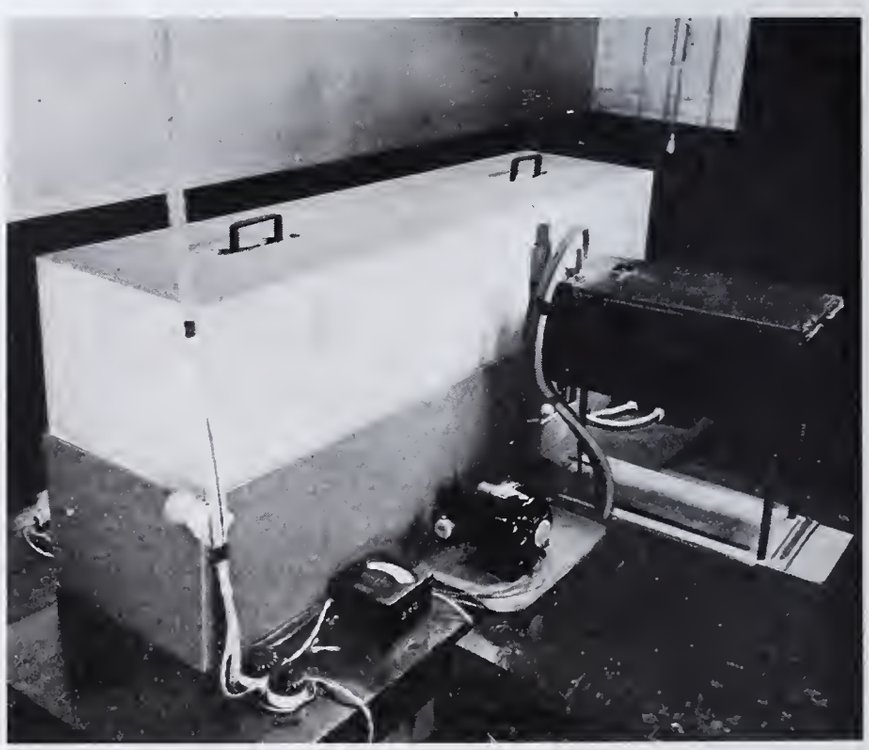

FIGURE 2 INFRARED SPECTROGRAPH

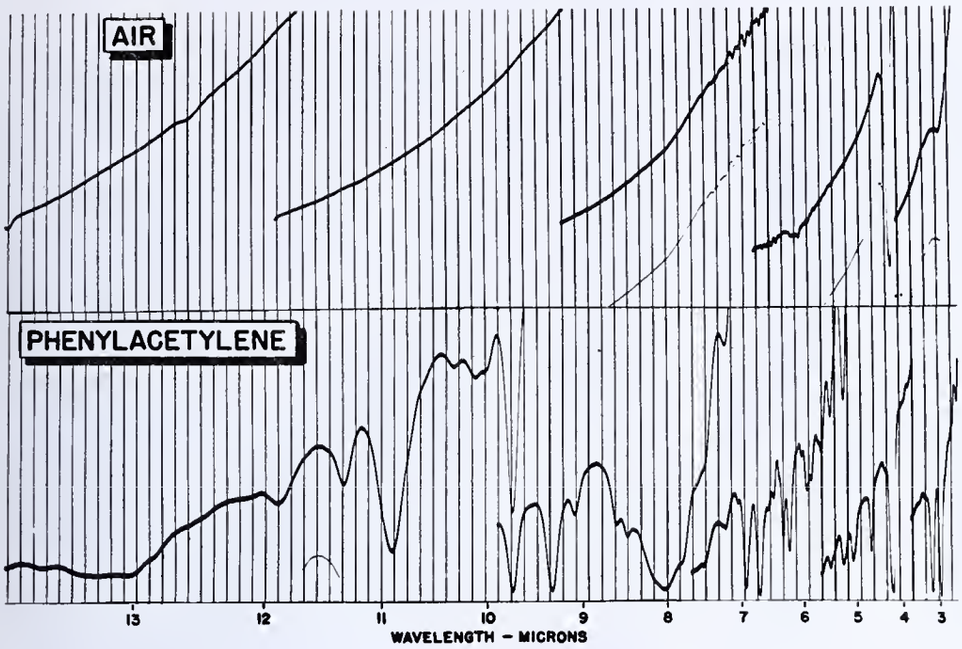

FIGURE 3. RECORDINGS OF INFRARED SPECTRA

The air spectrum (Figure 3) shows the absorption bands of carbon dioxide at 4.26 and at 13.9μ, as well as the saw-toothed, rotation-vibration band of water vapor extending from 5.5 to 7μ. A typical spectrum of an organic compound is that of phenylacetylene (Figure 3) in a liquid layer 0.1 mm. thick. The downward dips of the galvanometer indicate the absorption bands or regions of selective absorption characteristic of the phenylacetylene molecule.

Sample Preparation

Gases, liquids, and solids can be investigated by means of infrared spectroscopy. Examples of the various types of cells and sample preparations are shown in Figure 4.

Gases are placed in evacuable cells of glass having rock-salt windows; a convenient cell length for organic vapors has been found to be about 5 cm. with pressures of the vapor ranging from atmospheric down to 0.1 atmosphere. Organic liquids are observed in the form of thin layers in cells consisting of two carefully ground and polished rock-salt plates separated the proper distance by shims of lead or platinum. Cell thicknesses are usually 0.1 to 0.01 mm., but in special cases may be as great as 1 mm. or even larger. These cells, described in detail elsewhere (*5*), are constructed without the use of waxes and are sealed tightly enough to hold volatile liquids. Having both an entrance tube and orifice, they may be readily cleaned by forcing cleaning solvents through the cell, avoiding the troublesome necessity of dismantling the cell after each use.

Solids are usually observed in solution if suitable solvents can be obtained. Carbon disulfide and carbon tetrachloride are the most favored solvents because of their high infrared transmission. In some cases—e. g., the plastics—the spectra can be readily obtained by putting the sample into the form of a film having a thickness equivalent to those of the organic liquids mentioned. Solids for which no suitable solvents can be obtained and which cannot be put into film form may be studied in the form of thin layers of finely ground powder wet with carbon tetrachloride or carbon disulfide.

Identification of Unknowns

The first application of infrared spectroscopy in the analytical field to be considered is the identification of unknown organic compounds which are fairly free of impurities. Distinction is made here between identification and characterization. In the case of compounds not previously prepared or isolated, the infrared method offers very valuable aid in the identification of those groupings and linkages which produce characteristic infrared bans. In such cases it is not usually possible to derive the exact structure of the unknown compound.

A commoner problem, however, is the identification of compounds which are available in the pure state for comparison. Often the circumstances of a particular case provide clues as to the nature of the unknown, and a number of possible compounds may be suggested. It is then necessary merely to obtain and compare the spectrum of the unknown with the spectra of the suggested compounds. When a match is obtained the identification is complete and the problem is solved.

Too often, however, the suggested compounds turn out not to include the unknown. A general technique of identification then must be put into operation which involves the use of a catalog of infrared spectra. This catalog must contain the spectra of a large number of compounds and its usefulness improves as the number increases. The question of systematizing the spectra in such a catalog may depend somewhat

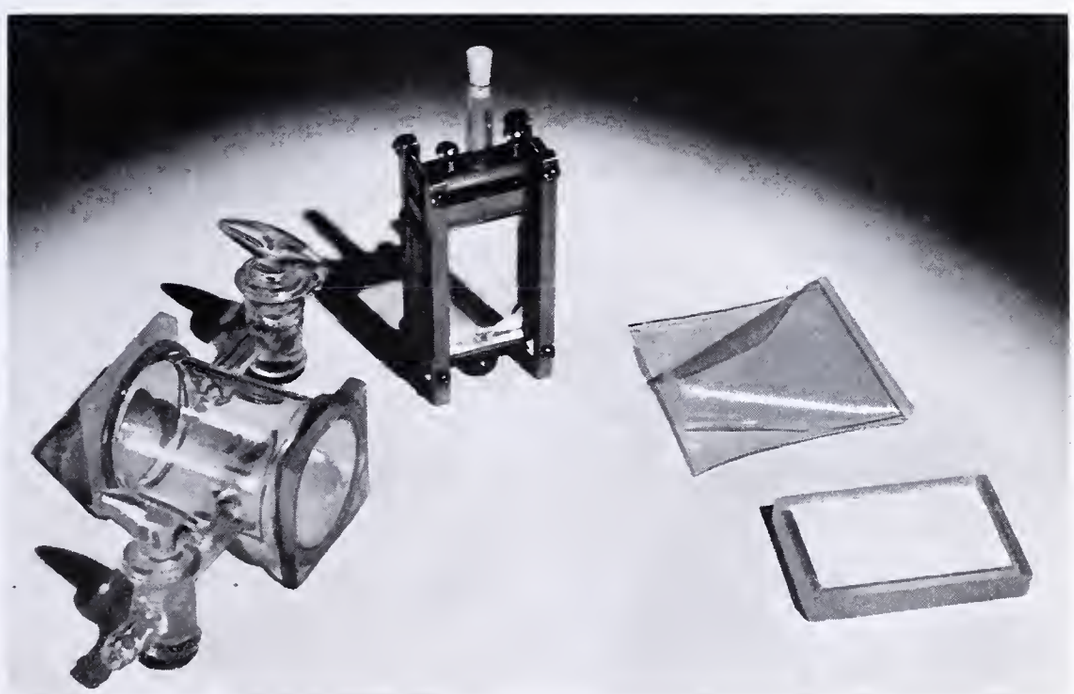

FIGURE 4. VAPOR AND LIQUID CELLS, PLASTIC FILM, AND POWDER LAYER ON ROCK-SALT PLATE

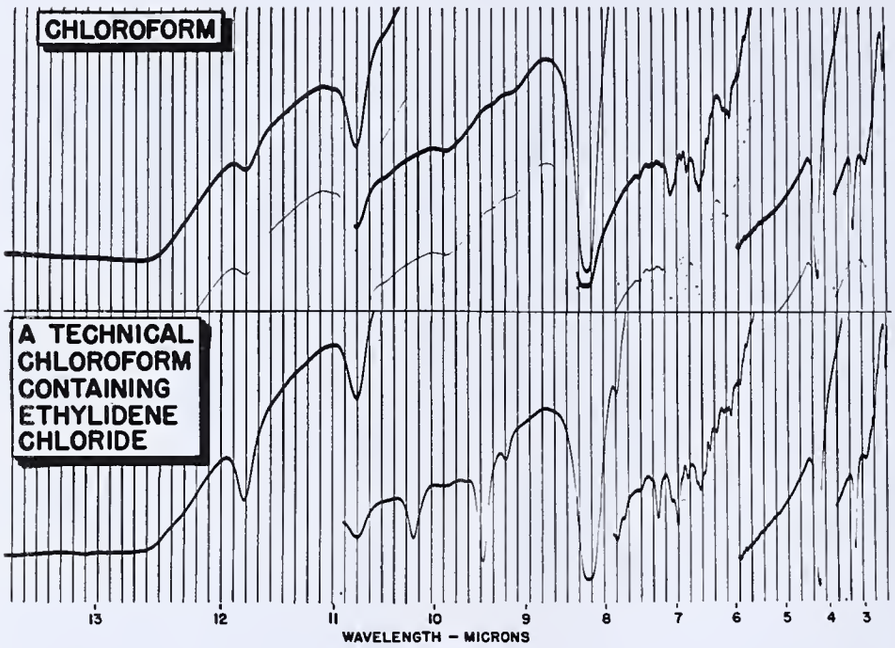

FIGURE 5. DETECTION OF ETHYLIDENE CHLORIDE IN CHLOROFORM

upon the type of problem most often encountered in a particular laboratory, but the following general methods have proved satisfactory.

Employing the method of Coblentz (*2*) for comparison of spectra, the absorption bands of a spectrum are represented as vertical lines plotted on a wave-length (or frequency) scale. Intensities of the absorption bands are indicated by the heights of the lines; for practical purposes these can be conventionalized to three different heights corresponding to strong, medium, or weak. The widths of very strong bands can be represented by correspondingly broad lines. In this manner the spectra of a large number of compounds can be conveniently plotted on an ordinary sheet of graph paper.

The spectra can be classified according to the presence in the compounds of the particular subgroups or linkages which produce characteristic infrared bands—for example, compounds containing O—H form one section of the catalog and those containing ≡C—H another, etc. Some repetition is, of course, inevitable. Another typical section of the catalog contains the spectra of the various chlorinated hydrocarbons arranged according to increasing chain length and increasing numbers of substituted chlorine atoms.

Another method of tabulating the spectra which appears to be of great practical value is to classify according to boiling points of the compounds.

In tracing down an unknown the procedure is to plot the spectrum on a strip of graph paper which can be compared directly with the spectra in the catalog. In this way a large number of compounds can be checked in a very brief time. After a match is found the original spectrum records are compared for final verification.

A third method of cataloging employs an index of wave lengths compiled in the manner adopted by Hanawalt and co-workers

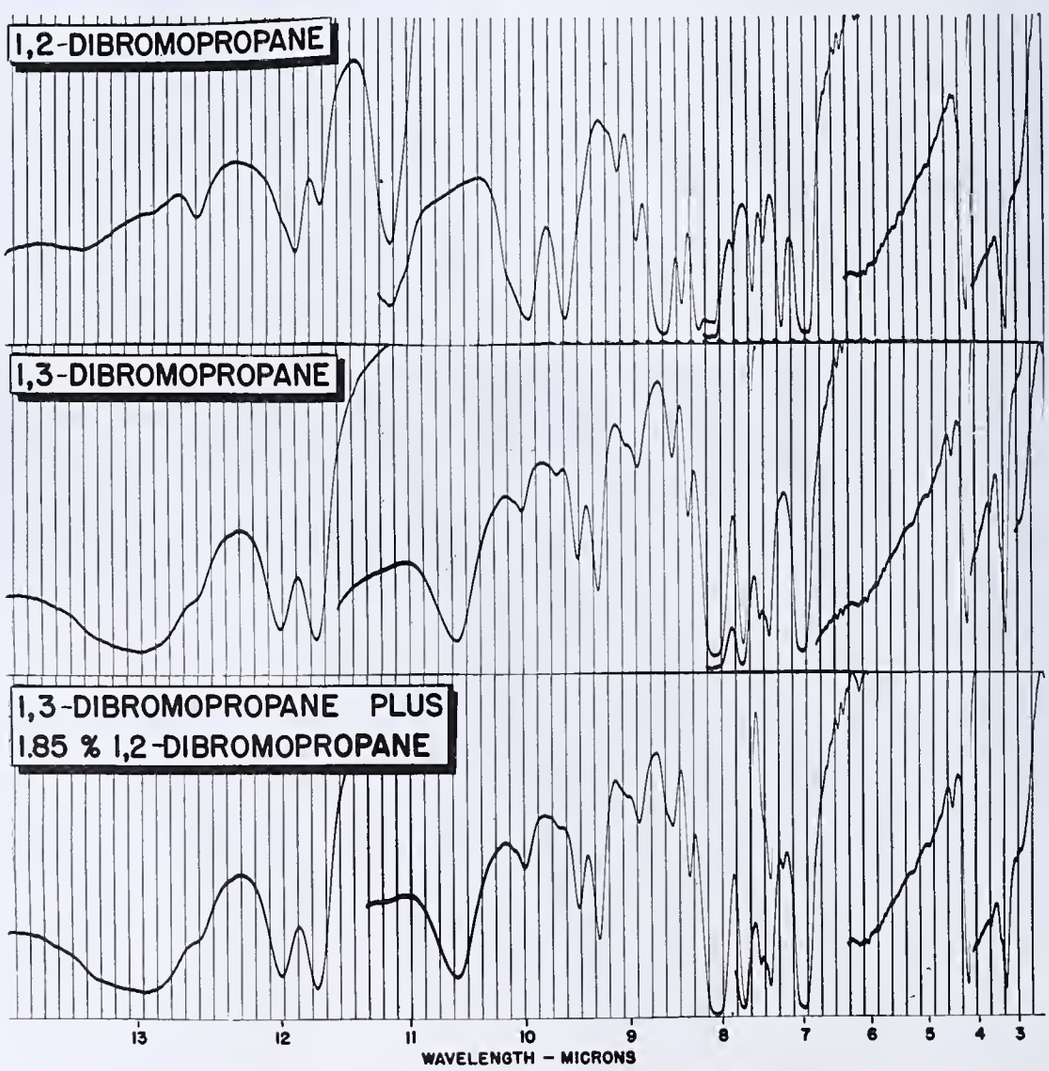

FIGURE 6. DETECTION OF 1,2 ISOMER AS IMPURITY IN 1,3-DIBROMOPROPANE

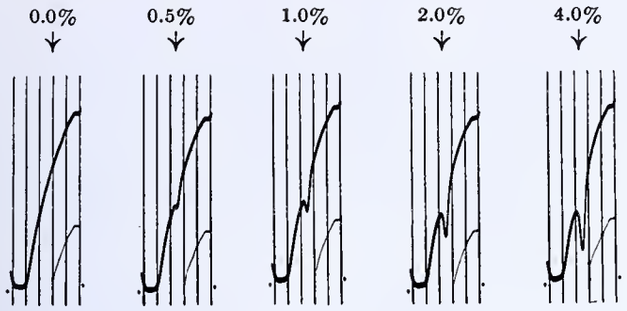

FIGURE 7. ADDITION OF METHYLENE CHLORIDE TO CHLOROFORM
Spectral interval from 8.35 to 7.50μ, and methylene chloride band at 7.93μ

(*6*, *7*) for x-ray spectra. The bands characteristic of subgroups and linkages are omitted in this index and use is made of the other bands which can be regarded as characteristic of the molecules as a whole. This obviates the difficulty of recording large numbers of compounds having strong bands at almost exactly the same wave length—for example, the band at 3.4μ of compounds with C—H groups.

Detection and Identification of Impurities

Detection and identification of impurities constitute a more difficult but much more important use of infrared spectroscopy. Fortunately the spectra of mixtures of organic compounds in gas, liquid, or solid state do not usually differ appreciably from a mere superposition of the spectra of the compounds taken individually. Cases where differences occur are those involving association, polymerization, or compound formation; in fact, these spectral differences are good criteria for such chemical behavior. Extremely small wave-length shifts are usually noticed when a compound is mixed with a highly polar solvent, but this offers no serious difficulty.

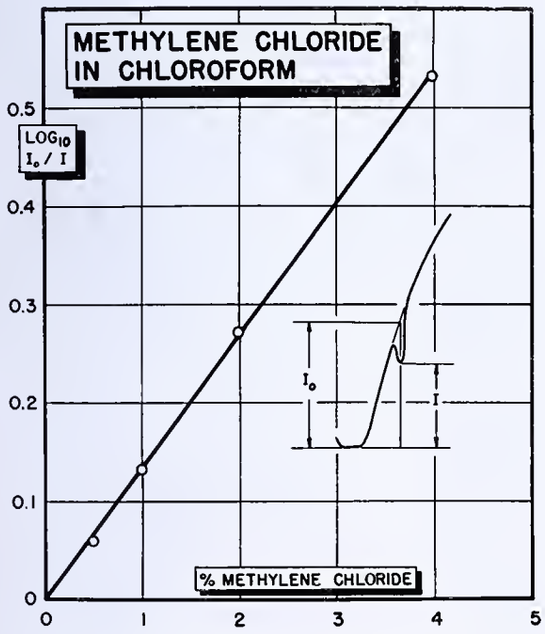

FIGURE 8. INFRARED MEASUREMENT OF METHYLENE CHLORIDE IN CHLOROFORM
Abscissa in per cent by volume

The usual method of testing for impurities is simply to compare the spectrum of the sample with that of a purified sample of the same compound. This is demonstrated in Figure 5 by the spectra of two samples of chloroform, one pure and the other showing plainly the superimposed absorption bands of an impurity of ethylidene chloride. When a pure sample of the compound under investigation is not available for comparison, the presence of impurity can often be detected through the use of partially purified samples which reveal the impurity by the weakening of its absorption bands. Fractional distillation is an enormous aid in this direction. Furthermore, impurity can sometimes be detected by use of the characteristic subgroup bands—e. g., the detection of an aldehyde impurity through the presence of a C=O band in a material not having this group.

Identification of impurities, although complicated by the background spectrum of the main constituent of the sample, is accomplished in much the same manner as already detailed for unknowns in a more refined state. Often many of the bands of the impurity are completely obscured by those of the main constituent and the comparatively few remaining bands must be relied upon for the identification. If only two or three of the absorption bands of the impurity can be picked out and these coincide exactly with bands of a suspected compound, and if all the strong bands of the suspected compound are accounted for as occurring in the spectrum or obscured by the bands of the main constituent, there can be little doubt as to the correctness of the identification. Further corroboration is given if the intensities of the matched absorption bands are correct.

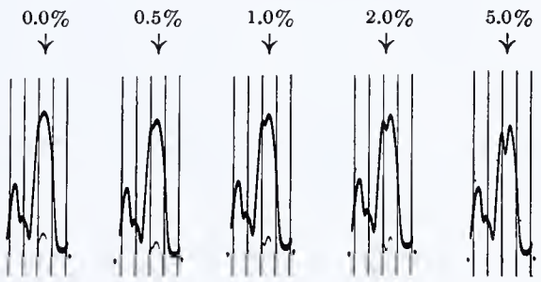

FIGURE 9. ADDITION OF 1,2-DIBROMOPROPANE TO 1,3-DIBROMOPROPANE
Spectral interval from 7.70 to 7.00μ, and 1,2-dibromopropane band at 7.29μ

Owing to the fact that fractional distillation is the commonest means of purification, the boiling points of the impurity and the main constituent are usually not far different. Reference to the catalog of spectra classified as to boiling points of the compounds is therefore an efficient means of search.

The view has often been expressed that successful analysis by means of infrared requires that the impurity compound possess characteristic groupings or linkages not present in the primary constituent. An example to the contrary is that of an impurity of 1,2-dibromopropane in 1,3-dibromopropane, the spectra of which are shown in Figure 6. Although these compounds are isomers differing only in geometrical structure, the infrared spectra are seen to be very different. Furthermore the 1,2 isomer can be detected in concentrations as low as 0.3 per cent in mixtures with 1,3-dibromopropane.

Quantitative Analysis

In addition to detection and identification of impurities, it has been possible in a large number of cases to develop accurate quantitative determinations of organic impurities by means of infrared spectra. The method consists first of selecting an absorption band of the impurity which does not fall too close to bands of the primary constituent of the mixture. This is illustrated in Figure 7, which shows a short wave-length interval of the spectra of samples of chloroform containing methylene chloride in amounts varying from 0 to 4 per cent (by volume). The cell used is 0.1 mm. thick. The absorption band of methylene chloride at 7.93μ is seen to increase in depth as the concentration increases.

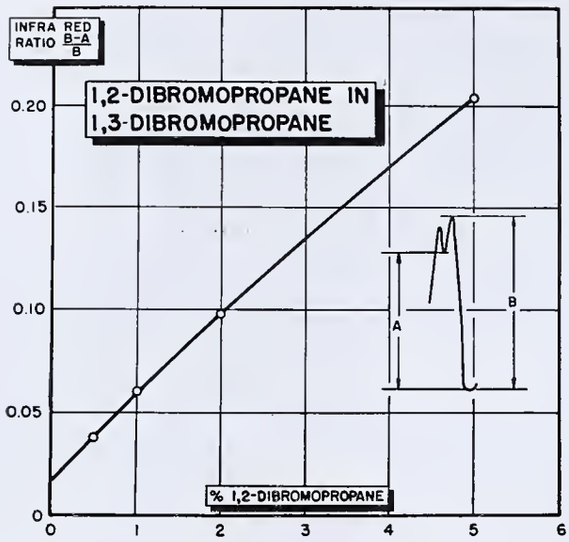

FIGURE 10. INFRARED MEASUREMENT OF 1,2-DIBROMOPROPANE IN 1,3-DIBROMOPROPANE

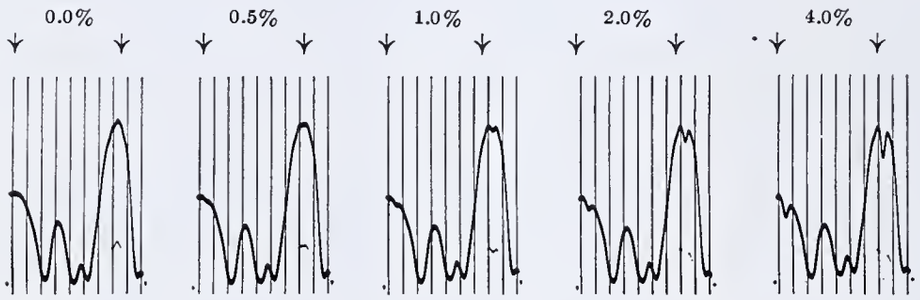

FIGURE 11. ADDITION OF PROPYLENE DICHLORIDE TO ETHYLENE DICHLORIDE
Spectral interval from 8.50 to 7.00μ, and propylene dichloride bands at 8.44 and 7.27μ

Recording of the spectra as here described in the form of transmitted radiation curves has led to very simple and accurate methods of measuring impurity bands. One method, used in the example of methylene chloride in chloroform, consists of measuring the intensities I_0 and I of the incident and transmitted radiation, respectively, at the wave length of the band. The value of I_0 is obtained by drawing in a straight line tangent to the spectrum curve at the position of the impurity band as shown in Figure 8. The values of $\log_{10} I_0/I$ (actually measured from the spectra of Figure 7) are then plotted against the concentration of methylene chloride in per cent by volume (Figure 8). The result is a straight line through the origin, attesting to the validity of Beer's law for this case. As a matter of fact, no deviation from Beer's law has been found in any of the many cases of organic liquid mixtures which have been encountered where no association occurs.

An analysis of a sample of chloroform for methylene chloride is therefore made in the following manner:

The sample, amounting to less than 0.1 cc., is placed in the cell and inserted in the beam of the radiation entering the spectrograph. The instrument then records the short interval of the spectrum shown in Figure 7. A measurement of $\log_{10} I_0/I$ is made from the record and the percentage of methylene chloride is read directly from the standard curve of Figure 8. The error of this determination amounts to about ± 0.05 per cent (of total sample) and the sensitivity is such that measurements can be made in concentrations of methylene chloride as low as 0.05 per cent.

In many cases of quantitative determination, a band of the impurity must be selected which is very close in wave length to bands of the primary constituent. Such a case is that of 1,2-dibromopropane in 1,3-dibromopropane which has already been mentioned (Figure 6). Figure 9 shows short intervals of the spectra of samples containing various percentages of the 1,2 isomer. Here again the cell thickness is 0.1 mm. The absorption band of the 1,2-dibromopropane at 7.29μ can be seen to deepen as the concentration increases from 0 to 5 per cent. The measurement made in this case is the entirely empirical deflection ratio indicated in Figure 10. A graph of this ratio plotted against percentage of the 1,2-dibromopropane yields the smooth curve shown in Figure 10.

For this type of measurement the spectrum need not be recorded but instead the galvanometer deflections are read visually on a scale while the instrument automatically scans the short wave-length interval covering the impurity band (Figure 9).

This analysis requires less than 5 minutes for completion from the receipt of the sample to the final reading of the percentage of 1,2-dibromopropane from the graph of Figure 10. The error of the determination is again about ± 0.05 per cent (of total sample) and a concentration as low as 0.3 per cent can be detected. The amount of sample required is less than 0.1 cc.

A similar type of deflection ratio is employed in the case of impurity of propylene dichloride in ethylene dichloride (Figure 11). In this case two absorption bands of propylene dichloride at 7.27 and 8.44μ can be seen. The deflection ratio used in the measurement of the 7.27μ band and the resulting curve against concentration of propylene dichloride are shown in Figure 12. The error of the determination is about ± 0.05 per cent (of total sample), and the sensitivity is 0.1 per cent. Time required for a complete analysis of a single sample is less than 5 minutes.

As in most methods of quantitative analysis, the infrared method may be complicated by the presence of several impurities; this happens if there is overlapping of the absorption bands. In a considerable number of instances, however, it has been possible to analyze quantitatively two or three different impurities in

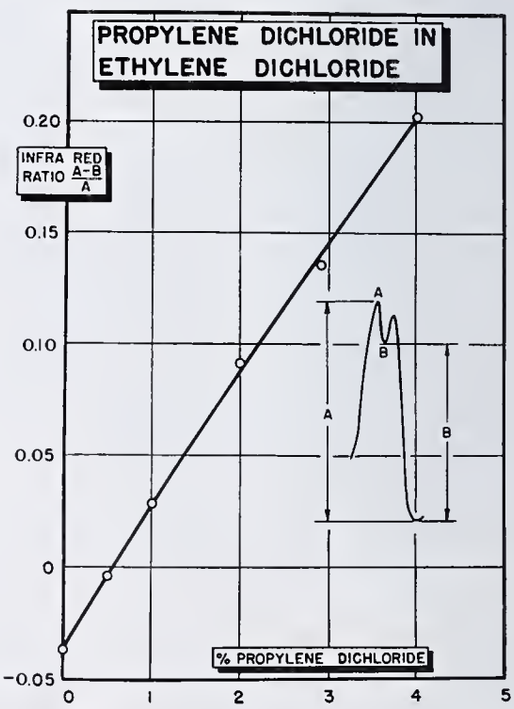

FIGURE 12. INFRARED MEASUREMENT OF PROPYLENE DICHLORIDE IN ETHYLENE DICHLORIDE

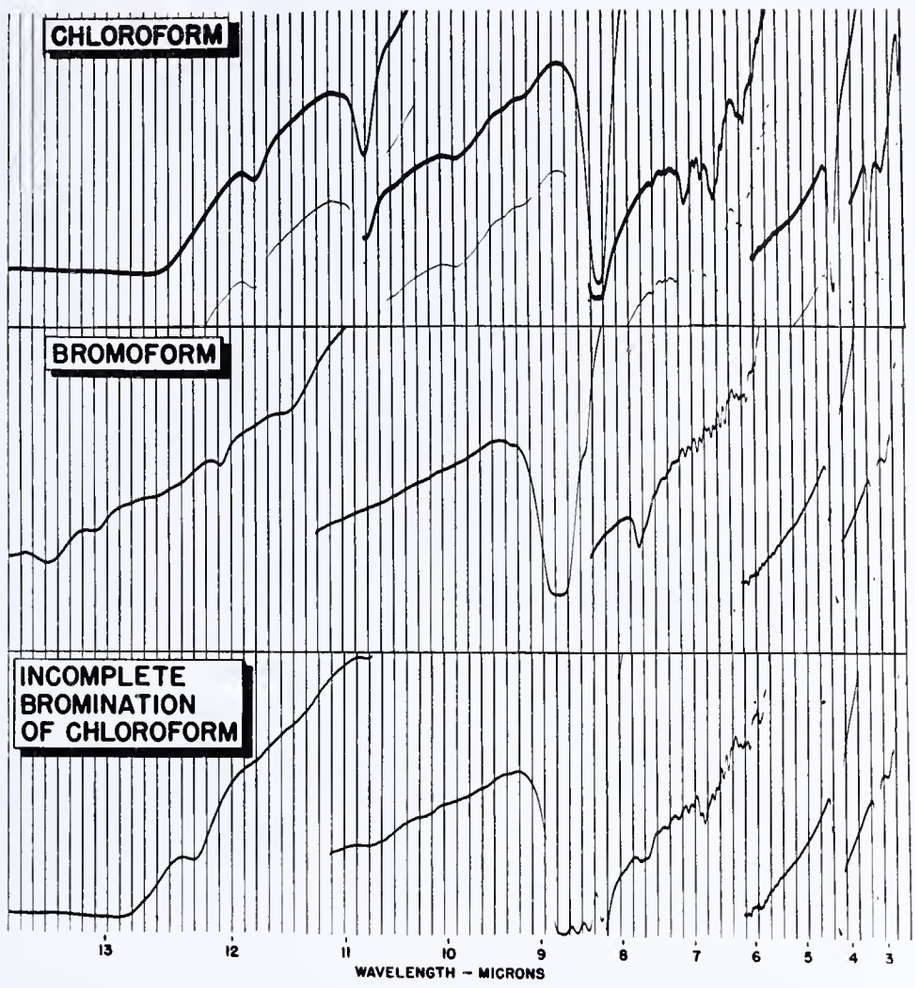

FIGURE 13. DETECTION OF INTERMEDIATES IN BROMINATION OF CHLOROFORM TO BROMOFORM

the same sample. An example is the determination of both methylene chloride and carbon tetrachloride in chloroform.

The examples of quantitative analysis mentioned and many others have been successfully handled by the instrument and methods described. The materials analyzed include both aliphatic and aromatic hydrocarbons, alkyl and aryl halides, alcohols, phenols, ethers, and others. The amount of sample required places the method in the microanalytical field; furthermore, the sample can be returned unchanged after an infrared analysis. Sensitivities of infrared determinations average from 0.1 to 0.05 per cent (by volume) as the minimum detectable concentration of impurity, and in some cases measurements can be made down to 0.001 per cent.

The described method of obtaining and measuring infrared spectra for quantitative analysis eliminates a number of possible errors. In the first place, no change in cell or cell position is made during a determination. All measurements are made at points of the spectrum sharply defined by the absorption pattern itself; this removes calibration errors which might arise should the method depend upon setting the instrument at definite wave lengths. Furthermore, the use of deflection ratios removes any effect of changes in instrument sensitivity, source intensity, or difference in adjustment of the external optical system. This also corrects for slight differences in cell transmission and for changes in general transmission of the samples caused by presence of free carbon, etc. Further removal of the last-mentioned effects is accomplished by making all measurements at points of the spectrum as close together as possible; incidentally, this allows only a few seconds of time to elapse between readings. Correction for a small amount of stray, short wave-length radiation in the instrument is made, as shown in Figures 8, 10, and 12, by using galvanometer zeros defined by near-by bands in which the absorption is total. This small correction is needed only at wave lengths greater than 6μ and can be furnished also by using glass in place of the metal shutter.

It may be argued that other more complicated methods of recording infrared spectra which yield percentage transmission or absorption curves directly (*4*) would be more suitable for quantitative determinations. The spectra shown in Figures 7, 9, and 11 demonstrate, however, that what is to be measured in determining an impurity is the superimposed absorption of a single sharp band of the impurity compound. For this purpose the recording of transmission or absorption percentages would at best give spectrum curves very little different from those of the present instrument, and identical measurements of the impurity band would have to be made.

Detection of Intermediates

A study of the reaction mechanism involved in the bromination of chloroform to bromoform affords a simple example of another use of infrared spectroscopy.

The question was the following: Does a molecule of chloroform brominate completely and at once to bromoform, or is the process one of successive replacement of the chlorine atoms with bromine atoms, forming first the intermediate compounds $CHCl_2Br$ and $CHClBr_2$? According to the first process if the bromination of a sample of chloroform is halted at an incomplete stage the reaction products should be chloroform and bromoform only; if the second mechanism takes place the intermediates should be present as well.

The answer to the question is clearly shown by the infrared spectra (Figure 13). Chloroform has a band at 8.24μ; the corresponding band of bromoform lies at the longer wave length, 8.74μ. The two intermediates should each have a band falling between those of chloroform and bromoform. The spectrum of the incompletely brominated sample of chloroform shows that the two intermediates are indeed present, two additional bands appearing between those of chloroform and bromoform. The process of bromination is therefore the second mentioned, one of successive replacement of chlorine atoms with bromine atoms.

Any discussion of applications of infrared spectroscopy would be incomplete without at least a brief comparison with the Raman effect. The two methods are similar in that the Raman spectra, although obtained by ordinary photographic spectrographs, correspond to molecular vibration frequencies as do the infrared spectra.

From the standpoint of success in practical applications of the kind discussed in the present paper, however, the two

methods differ considerably. The Raman effect has a definite advantage over infrared spectroscopy in being able to study materials in water solutions; but in the general organic field the infrared method is by far the more practicable. Important disadvantages of the Raman method are: (1) the necessity of refining each sample to remove ever-present traces of impurities which fluoresce, masking the spectra; (2) the long exposure times and general difficulty of producing spectra suitable to be microphotometered; and (3) the insensitivity of the method to compounds in small concentrations.

Advantages of analysis by means of infrared spectroscopy include the following: The amount of sample required is very small; no preliminary refinement of the sample is needed; and the method is sensitive to small concentrations, is accurately quantitative, and requires a very short time for a complete analysis.

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Rapid Determination of Antimony, Tin, and Bismuth

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New short methods for the accurate determination of antimony, tin, and bismuth in metals, alloys, and ores are described. Antimony, tin, and bismuth are coprecipitated with manganese dioxide formed by the reaction of manganese ion and potassium permanganate in hot dilute nitric or sulfuric acid solution.

For the determination of antimony, tin, or both, the manganese dioxide precipitate is fumed with sulfuric acid, potassium hydrogen sulfate, and ammonium sulfate. To determine antimony the solution is diluted with sulfur dioxide water, boiled down with hydrochloric acid, diluted, and titrated with potassium permanganate or potassium bromate. Tin is determined by reducing with nickel and hydrochloric acid the solution containing the fumed manganese dioxide precipitate, or the solution that remains after the titration of the antimony, and finally titrating with standard iodine solution.

For the determination of bismuth the manganese dioxide precipitate is dissolved in dilute nitric acid and hydrogen peroxide. The bismuth is separated with the help of zinc oxide, which precipitates the bismuth as basic nitrate. This in turn is dissolved in nitric acid, from which the bismuth is finally precipitated and weighed as bismuth oxychloride.

BLUMENTHAL (*1*) devised the coprecipitation of antimony with manganese dioxide by the reaction of manganous ion and potassium permanganate in hot dilute nitric or sulfuric acid solution. He states that tin and bismuth are also quantitatively precipitated, whereas arsenic comes down incompletely, and lead and copper are precipitated only in traces. Blumenthal confines the application of his method to the determination of antimony in copper. The authors have elaborated a considerably shorter method by omitting the removal of certain contaminations of the manganese dioxide precipitate. Many useful and time-saving applications are possible, facilitating considerably the determination of antimony, tin, and bismuth in various metals, alloys, and ores.

Determination of Antimony. A reliable and at the same time fast determination of antimony in blister copper has been investigated by the authors.

The methods of Jungfer (*16*) and Hampe (*15*), who remove the copper as cuprous iodide and thiocyanate, respectively, are very lengthy, involved, and too expensive for routine work. The method proposed by Evan (*9*), who separates the copper with sodium hypophosphite, has the same disadvantages. The coprecipitation of antimony oxide with metastannic acid, as described by Scott (*25*), is applicable only in the presence of large amounts of tin and is unreliable when iron is present, as stated by Classen (*5*). The method involving the principle of coprecipitating antimony with ferric hydroxide, advocated by Brownson (*4*), is too controversial to be considered a good standard method. Blumenthal's method (*1*), though unquestionably correct, as confirmed by Park and Lewis (*22*), and having many advantages over any of the methods mentioned above, is too lengthy for routine work. [Brownson's method is criticized adversely by Blumenthal (*1, 2*), who claims that antimony comes down only incompletely, and favorably by Boehm and Raetsch (*3*).]

This paper presents a very accurate method, so rapid that it requires no more than 3 to 4 hours as compared to about 7 hours by Blumenthal's method and more than one day by other methods. It has been successfully applied to various ores, making it possible to perform an antimony determination in ores in about 5 hours, whereas any of the established standard methods for the determination of antimony in ores takes more than one day.

Determination of Tin. The authors show that determination of tin with the aid of the manganese dioxide coprecipi-

tation is very useful, particularly when small amounts of tin must be separated from large quantities of copper, lead or silver which would either interfere or render a proper determination more difficult. A tin determination in blister copper takes about 4 hours and a simultaneous determination of tin and antimony requires about 5 hours.

Determination of Bismuth. In separating bismuth from copper, lead, silver, and other impurities the authors have made use of the manganese dioxide coprecipitation. Where it is customary and advisable to scorify a certain amount of the sample [Craig (*8*) advocates scorification for the separation of bismuth from antimony and other impurities], the manganese dioxide precipitate is contaminated by only small amounts of lead. In such cases the final procedure for the determination of bismuth as bismuth oxychloride has been considerably shortened by the authors by eliminating the customary sulfide, carbonate, and basic nitrate separations and substituting a new separation of bismuth from lead and manganese which is based upon the fact that zinc oxide precipitates bismuth as basic nitrate while lead and manganese stay in solution. The final bismuth oxychloride precipitate can be obtained in less than 6 hours.

Preparation and Solution of Sample

In dissolving the sample the following facts were kept in mind:

The coprecipitation of antimony, tin, and bismuth with manganese dioxide is best performed in nitric acid solution. A sulfuric acid solution was used by the authors for the determination of antimony and tin when previous decomposition of the sample with aqua regia was required and when the sample was free from or low in lead.

The acidity is very important for the precipitation of the bismuth, because it should not exceed 0.07 *N*—that is, 1.5 ml. of concentrated nitric acid in 300 ml. of solution—otherwise precipitation of bismuth will be incomplete. The acidity may be as high as 0.5 *N* without running the risk of losing antimony or tin.

Bullion and Alloys of Copper, Silver, Lead, and Zinc. An adequate amount of the metal was dissolved in nitric acid, disregarding any undissolved oxides of antimony or tin. The solution was warmed to expel any oxides of nitrogen and the acidity was adjusted.

Ores and Concentrates. To determine antimony and tin in ores and concentrates, and bismuth in high-grade bismuth ores, an adequate amount of the sample was fused with sodium peroxide in a nickel crucible. The fusion was leached with water and acidified with nitric acid. A little hydrogen peroxide was added if the solution did not become clear at this stage. Any silica or undissolved oxides of antimony or tin were disregarded. The excess of hydrogen peroxide was expelled and the acidity adjusted.

To determine bismuth in low-grade bismuth ores (bismuth below 1 per cent), 2.5- to 5-gram portions of the sample were scorified with 65 grams of test lead. When the amount of bismuth was very low 2 to 4 of the lead buttons, which weighed about 25 grams, were combined, scorified again to about 25 grams, flattened, and dissolved in dilute nitric acid. The acidity was adjusted to 0.07 *N*.

Experimental Procedure

To about 300 ml. of the carefully adjusted nitric or sulfuric acid solution containing not more than 300 mg. of antimony, tin, or bismuth, 5 ml. of about 5 per cent manganese sulfate solution were added. [In the presence of considerable amounts of lead and always for the determination of bismuth, instead of manganese sulfate a manganese nitrate solution of corresponding strength was used.] The solution was heated to boiling and 5 ml. of about 1 *N* potassium permanganate were added with the aid of a pipet. After boiling for about 3 minutes, 3 ml. more of the potassium permanganate solution were added. The solution was allowed to simmer for 3 more minutes and then was filtered through a quick-running filter paper and washed twice with hot water. The filtrate was transferred into the original beaker, and 3 ml. of 5 per cent manganese sulfate solution were added. The solution was brought to a boil, and the precipitation of manganese dioxide was repeated with 3 ml. of 1 *N* potassium permanganate solution. After boiling for about 3 minutes, the precipitate was filtered through the original paper and washed 8 to 10 times with hot water.

Determination of Antimony. The beaker was wiped with a small piece of paper moistened with sulfur dioxide water. This, together with the manganese dioxide precipitate and the filter paper, was placed in a 500-ml. Erlenmeyer flask, to which were added 3 grams of ammonium sulfate, 1 gram of potassium hydrogen sulfate, and 25 ml. of concentrated sulfuric acid. The precipitate was fumed on a hot plate, first cautiously and then with the full heat, until all the carbon was oxidized and the faint pink color of the manganese sulfate prevailed. This operation was performed very rapidly because of the oxidizing effect of the manganese dioxide. A little solid ammonium persulfate was added and the fuming continued over a bare flame for about 2 more minutes.

After the solution had cooled, 75 ml. of sulfur dioxide water and 100 ml. of concentrated hydrochloric acid were added, and the solution was boiled down to about 85 ml. Next, 200 ml. of cold water were added and the solution was cooled to below 15° C. and finally titrated with 0.1 *N* or weaker potassium permanganate solution. To carry out the titration with potassium bromate, 120 ml. instead of 100 ml. of concentrated hydrochloric acid were added, and the solution was boiled down to about 100 ml., diluted with 100 ml. of hot water, and titrated using methyl orange as indicator.

$$5Sb_2(SO_4)_3 + 4KMnO_4 + 16H_2SO_4 = 5Sb_2(SO_4)_5 + 2K_2SO_4 + 4MnSO_4 + 16H_2O$$

$$3SbCl_3 + KBrO_3 + 6HCl = 3SbCl_5 + KBr + 3H_2O$$

Determination of Tin. When the potassium permanganate method was employed for the determination of antimony, the solution remaining after the titration was evaporated to about 200 ml., this evaporation being unnecessary when potassium bromate titration was used. In case a previous antimony determination was not required, the solution containing the fumed manganese dioxide precipitate was diluted to 200 ml. To the 200 ml. of solution obtained by any of the above methods, 60 ml. of concentrated hydrochloric acid were added. The tin was reduced by boiling with nickel strips or foil, after which the solution was cooled under carbon dioxide and titrated with a standard iodine solution using starch as indicator.

$$SnCl_2 + I_2 + 2HCl = SnCl_4 + 2HI$$

Determination of Bismuth. *Scorification Employed, or Sample Free from Antimony, Tin, and Iron.* The paper with the manganese dioxide precipitate was unfolded and the precipitate was washed back into the original beaker, in which it was dissolved with 30 ml. of a mixture of equal volumes of 15 *N* nitric acid, 3 per cent hydrogen peroxide, and water. The solution was boiled for about 10 minutes to destroy the excess hydrogen peroxide, and was then diluted to 120 ml. Ammonia was now added to neutralize all but a small amount of the nitric acid. When a brown coloration developed during the neutralization, due to the formation of manganese hydroxides, a little sodium nitrite was added until the solution became clear. At this point about 0.5 gram of sodium nitrite and an excess of zinc oxide were added. There should be an excess of about 0.75 gram of zinc oxide after all the bismuth had precipitated and the solution became cloudy.

After boiling for about 1 minute the precipitate was filtered through a No. 42 Whatman or corresponding filter paper and washed 5 times with hot water. The precipitate was washed back into the original beaker, in which it was dissolved in 5 ml. of 15 *N* nitric acid and diluted to 100 ml. The zinc oxide precipitation was repeated as described above. The precipitate was then filtered through the original paper, washed 5 times with hot water, and dissolved in dilute nitric acid. The solution was warmed, filtered through the original paper, and washed with hot dilute nitric acid. To the solution 15 *N* ammonia was added to neutralize the greater part of the nitric acid. (When the amount of bismuth was very low the authors evaporated the nitric acid solution to dryness and took it up with a few drops of nitric acid and water. Ammonium chloride was added and the solution diluted to 600 ml.)

Finally 3 *N* ammonia was added drop by drop until the first sign of an opalescence was observed. At this stage 2 grams of ammonium chloride were added and the solution was diluted to about 600 ml. with hot water. The bismuth oxychloride was allowed to settle on a hot plate or a water bath, and finally filtered through a weighed Gooch crucible and washed with hot water. The precipitate was dried at 100° C. and weighed as

Table I. Determination of Antimony

Cu Used	Sb Used	Sb Found by $KMnO_4$ Method		Sb Found by $KBrO_3$ Method	
		Original	Duplicate	Original	Duplicate
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
3	10.0	10.1	10.0	9.9	10.1
5	10.0	9.9	9.8	10.2	10.0
5	50.0	49.8	50.0	49.9	50.0
10	10.0	10.0	10.0	9.9	9.7
10	100.0	99.7	100.2	99.6	100.0
15	100.0	99.6	99.8	99.5	99.6
25	50.0	49.3	49.0	49.3	49.0
25	100.0	98.8	98.2	97.4	97.8
50	50.0	46.9	47.3	46.8	46.1
50	100.0	92.1	93.7	89.7	90.4

bismuth oxychloride. The authors used the theoretical factor for small amounts of bismuth, and for larger amounts they established empirical factors as described by Craig (*7*).

No Scorification Employed and Sample Contained Antimony, Tin, or Iron. The manganese dioxide precipitate was dissolved in dilute hydrochloric acid and hydrogen peroxide. After the excess of hydrogen peroxide had been expelled by boiling, the solution was filtered, the acidity was adjusted to 0.5 *N*, and hydrogen sulfide was passed in. The precipitate was filtered and washed with hydrochloric acid–hydrogen sulfide. In case antimony or tin was present, the precipitate was treated with $(NH_4)_2Sx$ solution. The bismuth sulfide precipitate was dissolved in dilute nitric acid and filtered through the original paper. At this stage one zinc oxide separation was made as described in the preceding paragraph, omitting the addition of sodium nitrite, and bismuth oxychloride was precipitated as described.

Table II. Copper Occluded

Cu Used	Sb Used	Cu Found	
Grams	*Mg.*	*Mg.*	*Mg.*
5	12.5	1.9	2.1
10	25.0	3.3	3.0
25	50.0	6.7	7.0
50	65.0	8.1	8.1
25	100.0	8.3	8.7
50	100.0	8.9	9.2
25	300.0	9.1	8.9
50	300.0	10.0	9.8

Nature of Interference

Determination of Antimony. It is evident that the manganous salts formed by the reaction of manganese dioxide with sulfuric acid will not interfere with the proper determination of antimony, with either the potassium permanganate or the potassium bromate method as described in this paper. On the contrary, the manganous salts tend to prevent a possible reaction of the potassium permanganate with the excess hydrochloric acid. [Zimmermann (*29*), Reinhardt (*23*), Kessler (*17*), and Scott (*28*) used manganese sulfate as preventive solution for the determination of iron.] The manganese dioxide coprecipitates besides antimony, tin, and bismuth a part of the arsenic and traces of copper and lead. (However, lead will be precipitated to a considerable extent from a solution containing 25 grams of lead if the acidity amounts to only 0.07 *N*.) Lead and bismuth have no influence upon the determination of antimony by either method. The arsenic is expelled as volatile arsenic trichloride by boiling the solution which has previously been acidified with hydrochloric acid and reduced with sulfur dioxide water. The tin is in the oxidized state after the fuming. It is not reduced by sulfur dioxide, and as a consequence it will not influence the titration of the antimony.

There are contradictory statements in the literature concerning the nature of interference of copper in the determination of antimony. Low (*18*) and Lunge-Berl (*20*) state that in the presence of copper high results are obtained with the bromate method. "Ausgewählte Methoden" (*10*) advocate the opposite point of view—namely, that in the presence of copper low results are obtained with both methods, particularly with the bromate method. Scott (*24*) and Oesterheld-Honegger (*21*) state that copper has no influence at all when present in amounts not exceeding 150 mg.

To determine the exact nature of interference, varying amounts of C. P. antimony and copper were fumed with 25 ml. of concentrated sulfuric acid, 1 gram of potassium hydrogen sulfate, and 3 grams of ammonium sulfate. The procedure after the fuming was exactly the same as that described in this paper.

The results are given in Table I. It is evident that, using sulfur dioxide water as described in this paper, up to 10 mg. of copper have no effect upon the proper determination of antimony by either the potassium permanganate or the potassium bromate method. When the amount of copper exceeds 15 mg. low results are obtained with both methods.

To ascertain how much copper is occluded by the manganese dioxide precipitate under extreme conditions, varying amounts of pure copper to which varying amounts of C. P. antimony were added, were dissolved in nitric acid. The solution was diluted to 300 ml. and manganese dioxide was precipitated as described in the procedure. The copper was determined in the manganese dioxide precipitate, after the latter had been filtered and washed with hot water. Copper was never found to exceed 10 mg., as shown by Table II.

Iron present in considerable amounts (1 per cent or more) tends to contaminate the manganese dioxide precipitate.

In such cases the authors did not fume the manganese dioxide precipitate; instead they dissolved it with hydrochloric acid and hydrogen peroxide, expelled the excess hydrogen peroxide by boiling, adjusted the acidity to 0.5 *N*, and passed in hydrogen sulfide. The precipitate was filtered and washed free from iron and hydrochloric acid with sulfuric acid–hydrogen sulfide wash. The precipitate together with the paper was fumed exactly as the manganese dioxide precipitate was fumed, and the procedure was continued as described.

Determination of Tin. Manganous salts, iron, lead, and bismuth are obviously without any influence. Antimony will interfere only when present in large amounts. In such cases it was removed with iron nails or powder. The small amounts of copper which may be encountered in an analysis of copper bullion do not interfere.

Determination of Bismuth. *Scorification Employed, or Sample Free from Antimony, Tin, or Iron.* Tin and antimony which, when present in appreciable quantities, would interfere in the determination of bismuth as bismuth oxychloride, are for all practical purposes removed by the scorification. To ascertain how much tin and antimony are still present after one or two scorifications, varying amounts of tin and antimony were scorified with 65 grams of test lead, and were determined in the lead button. The results are presented in Table III.

Table III. Effect of Scorification

Sb Used	Sb Found		Sn Used	Sn Found	
	First Scorification	Second Scorification		First Scorification	Second Scorification
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
5	..	...	5	..	...
10	..	...	10	..	...
25	0.3	...	25	..	...
50	0.8	...	50	0.4	...
75	1.2	...	75	0.6	...
100	1.7	Trace	100	1.0	...
200	3.8	Trace	200	2.6	Trace
500	8.9	Trace	500	5.3	Trace

Table IV. Occlusion of Lead

Bi Used	Pb Used	Series A, Pb Found in MnO_2 Ppt.		Series B, Pb Found in ZnO Ppt.	
		Original	Duplicate	Original	Duplicate
Mg.	*Grams*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
10	20	125.3	122.8	2.2	2.7
10	30	152.2	160.3	3.1	3.1
25	20	140.7	148.5	2.9	3.4
25	25	149.4	146.4	3.7	3.5
25	30	167.8	179.4	4.1	4.9
50	20	169.5	163.7	4.5	4.8
50	30	190.2	199.9	5.2	5.8
100	30	222.3	230.7	7.1	6.4
100	20	204.9	211.3	6.2	6.3

The amounts of antimony and tin which remain after scorification, even under extreme conditions, will not interfere with the proper determination of bismuth as oxychloride.

As considerable amounts of lead will interfere, the authors carried out two series of tests to ascertain how much lead is occluded by the manganese dioxide and zinc oxide precipitates under extreme conditions. In series A varying amounts of bismuth and lead were dissolved in nitric acid, and two manganese dioxide separations were performed as shown in the procedure. The manganese dioxide precipitate was dissolved in hydrochloric acid and the lead was finally determined as the chromate. In series B the procedure was exactly as described in the method. The zinc oxide precipitate was dissolved in hydrochloric acid and the lead was finally determined as the chromate.

Table IV shows that only traces of lead reach the final solution in which the bismuth is precipitated as bismuth oxychloride. Traces of lead have no effect upon the proper determination of bismuth, as stated by Scott (*26*). Zinc will not contaminate the bismuth oxychloride precipitate.

To prove this, varying amounts of bismuth and 0.75 gram of zinc oxide were dissolved in nitric acid. The excess acid was neutralized with ammonium hydroxide and the bismuth was precipitated as bismuth oxychloride. This precipitate was filtered, washed with hot water, and dissolved in hydrochloric acid. The acidity was adjusted to 0.5 *N* and hydrogen sulfide was passed in. The zinc was determined in the filtrate from the bismuth sulfide and was never found to exceed 0.3 mg.

No Scorification Employed and Sample Contained Antimony, Tin, or Iron. Antimony, tin, and iron are removed by the procedure involving precipitation of the bismuth as sulfide. The influence of lead and zinc has been discussed above.

Corroboration and Verification

Determination of Antimony, Tin, and Bismuth. Varying quantities of antimony, tin, and bismuth have been determined with the methods described above by decomposing known amounts of these metals in nitric acid, diluting to 300 ml., adjusting the acidity, and proceeding as described in "Experimental Procedure". Generally the determinations were made separately, though, in some cases (marked A, B, C) antimony and tin were determined simultaneously or in the presence of each other. The accuracy of the methods is indicated by Table V.

The authors in addition have analyzed various kinds of samples by the methods described in this paper and compared the results with those obtained by standard methods (Table VI).

Table V. Accuracy of Results

Sb Used	Sb Found: $KMnO_4$ Method Original	$KMnO_4$ Method Duplicate	$KBrO_3$ Method Original	$KBrO_3$ Method Duplicate
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
5.0	5.1	5.0	5.1	5.0
25.0	24.9	25.3	24.8	24.7
50.0	50.3	49.7	49.8	50.0
100.0	99.5	100.0	99.6	100.2
200.0	200.5	199.9	199.8	200.1
Simultaneous Determinations (See A, B, C Below)				
(A) 50.0	50.0	49.8	49.7	49.9
(B) 100.0	99.8	99.6	100.2	99.6
(C) 100.0		Sb not determined		

Sn Used	Sn Found: Original	Duplicate	Triplicate
Mg.	*Mg.*	*Mg.*	*Mg.*
10.0	9.9	10.2	10.0
25.0	24.8	25.0	25.0
50.0	50.4	50.1	50.2
100.0	99.7	99.2	100.3
250.0	249.5	249.9	250.4
Simultaneous Determinations (See A, B, C Below)			
(A) 100.0	100.3	99.6	99.9
(B) 100.0		Sn not determined	
(C) 249.5	249.7	250.3	250.0

Bi Used	Bi Found: Original	Duplicate	Triplicate
Mg.	*Mg.*	*Mg.*	*Mg.*
5.0	4.8	4.9	4.8
25.0	25.2	24.9	24.6
50.0	49.5	49.8	50.2
100.0	100.2	100.0	99.7
200.0	199.7	200.3	199.7

Table VI. Comparative Analyses

Kind of Sample	Analyzed for	Found by Author's Method %	Found by Standard Method %	Standard Method Described by
Blister copper	Sb	0.27, 0.27	0.26, 0.26	Jungfer (*16*)
	Sb	0.26, 0.27	0.25, 0.26	Brownson (*4*)
	Sb	.. ..	0.27, 0.27	Blumenthal (*1*)
Average of 24 samples of blister copper	Sb	.. 0.33	.. 0.31	Brownson (*4*)
Bronze	Sb	0.04, 0.04	0.04, 0.04	Scott (*25*)
Pb, Cu, Sb ore	Sb	12.27,12.32	12.26,12.30	"Ausgewählte Methoden" (*11*)
Pb, Cu, Sb ore	Sb	10.22,10.18	10.16,10.20	"Ausgewählte Methoden" (*11*)
Scrap lead	Sb	4.12, 4.13	4.15, 4.12	Györy (*14*)
Blister copper	Sn	0.07, 0.07	0.07, 0.07	Classen (*6*)
Brass	Sn	0.72, 0.71	0.71, 0.71	Classen (*6*)
Pb, Sn, Cu ore	Sn	9.67, 9.71	9.66, 9.70	"Ausgewählte Methoden" (*13*)
Blister copper	Bi	0.05, 0.05	0.05, 0.05	"Ausgewählte Methoden" (*12*)
Blister copper	Bi	0.16, 0.17	0.16, 0.16	"Ausgewählte Methoden" (*12*)
Pb bullion	Bi	0.04, 0.04	0.04, 0.04	Scott (*27*)
Pb ore	Bi	0.64, 0.64	0.64, 0.63	Low (*19*)

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Determination of Biochemical Oxygen Demand

Comparative Study of Azide and Rideal-Stewart Modifications of Winkler Method

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IN THE determination of dissolved oxygen by the Winkler method, the azide modification for the destruction of nitrites has recently occasioned great interest. In a previous paper (*6*) a comparison was drawn between dissolved oxygen (D. O.) determinations made with the azide and the Rideal-Stewart modifications. No results on biochemical oxygen demand (B. O. D.) were reported at that time. The azide, in the previous study, was used separately in acid medium—that is, it was simply substituted for the potassium permanganate and oxalate used in the Rideal-Stewart procedure. Since then Barnett and Hurwitz (*2*) have recommended returning to the original Alsterberg procedure (*1*, *3*)—that is, adding the azide with the alkaline iodide reagent. This method is now used by the Sanitary District of Chicago (*4*). Either technique has an advantage in time required and simplicity over the Rideal-Stewart procedure. The background of the method has been ably reviewed in the previous papers and merits no further discussion here. Meanwhile, considerable data relative to the use of the various azide procedures and to the determination of B. O. D. by the azide modification have accumulated in the authors' laboratory; they present excellent confirmation of the value of this worthwhile method.

RIDEAL-STEWART. To the sample in a 300-ml. bottle add 0.7 ml. of concentrated sulfuric acid followed by enough permanganate solution (6.32 grams per liter) to obtain a violet tinge which persists for 5 minutes after shaking. Destroy the excess with a minimum amount of a 2 per cent potassium oxalate solution. When perfectly colorless add 1 ml. of manganous sulfate solution (480 grams of manganous sulfate tetrahydrate per liter) and 3 ml. of alkaline iodide solution (500 grams of sodium hydroxide and 150 grams of potassium iodide). Shake the sample for 20 seconds, allow the precipitate to settle and then acidify with 2 ml. of concentrated sulfuric acid. Titrate the liberated iodine on a volume of solution equivalent to 200 ml. of the original sample immediately with 0.025 *N* sodium thiosulfate, using starch indicator.

ALSTERBERG. The sodium azide used is combined with the alkaline iodide reagent. Alsterberg used 5 grams per liter. Barnett 8 grams per liter. Add 2 ml. of manganous sulfate and 2 ml. of the alkaline iodide–azide solution to the sample and shake for 20 seconds. Allow the precipitate to settle and acidify the sample with 2 ml. of concentrated sulfuric acid. Complete the titration as above.

MODIFIED AZIDE. Add 0.7 ml. of concentrated sulfuric acid to the sample as in the Rideal-Stewart procedure, followed by 0.8 ml. of a 2 per cent aqueous sodium azide solution. Stopper the bottle, mix the contents by shaking, and allow the bottle to stand for 10 minutes. Add 1 ml. of manganous sulfate and 3 ml. of alkaline iodide, and complete the determination exactly as stated in the Rideal-Stewart procedure.

Comparative B. O. D. Determinations by Modified Azide and Rideal-Stewart Methods

Comparisons were made at two laboratories. The samples from the upper Ohio River were analyzed on the laboratory boat *Kiski*, stationed at Dam 29 near Ashland, Ky. The Ohio River tributaries included in this area are the Scioto, Kanawha, Guyandot, Little Sandy and Big Sandy Rivers, and Tygert Creek. The remainder of the samples were examined at the main laboratory in Cincinnati. These included samples from the Cincinnati section of the Ohio River and the following tributaries: Little Miami, Great Miami, Turtle Creek, Todd's Fork, Kentucky, Whitewater, South Licking, and Licking. The sampling period included an interval of 11 months.

The data obtained have been divided into three groups corresponding to the B. O. D. ranges 0 to 2, 2 to 4, and above 4.0 p. p. m., and are presented in Table I. This classification was undertaken to determine the effect, if any, of increasing

TABLE I. COMPARATIVE DISSOLVED OXYGEN AND B. O. D. DATA

(Obtained by Rideal-Stewart and azide modifications upon 1396 Ohio River and tributary samples)

B. O. D. Range	Source of Samples	Number of Samples	Mean Dissolved Oxygen Initial		Mean Dissolved Oxygen Final		B. O. D.		Deviations, Azide minus Rideal-Stewart		
			Azide	Rideal-Stewart	Azide	Rideal-Stewart	Azide	Rideal-Stewart	Initial D. O.	Final D. O.	B. O. D.
P. p. m.			*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
0 to 2.0	Ohio River above Dam 29[a]	253	8.37	8.24	7.28	7.17	1.09	1.07	0.13	0.11	0.02
	Ohio River below Dam 29[b]	310	8.34	8.21	7.21	7.14	1.13	1.07	0.13	0.07	0.06
	Ohio River Cincinnati pool[c]	85	8.71	8.51	7.02	6.87	1.69	1.64	0.20	0.15	0.05
	Tributaries Cincinnati area[d]	269	8.21	8.06	7.16	7.04	1.05	1.02	0.15	0.12	0.03
	Tributaries Ashland area[e]	112	8.74	8.61	7.58	7.46	1.16	1.15	0.13	0.12	0.01
Average of all in B. O. D. range		1029	8.39	8.24	7.24	7.13	1.15	1.11	0.15	0.11	0.04
2.01 to 4.00	Ohio River above Dam 29	16	8.01	7.84	5.74	5.64	2.27	2.20	0.17	0.10	0.07
	Ohio River below Dam 29	29	7.99	7.81	5.63	5.61	2.36	2.20	0.18	0.02	0.16
	Ohio River Cincinnati pool	129	8.58	8.36	5.92	5.79	2.66	2.57	0.22	0.13	0.09
	Tributaries Cincinnati area	37	8.14	7.88	5.56	5.46	2.58	2.42	0.26	0.10	0.16
	Tributaries Ashland area	83	8.61	8.29	5.68	5.45	2.93	2.84	0.32	0.23	0.09
Average of all in B. O. D. range		294	8.44	8.20	5.77	5.63	2.67	2.57	0.24	0.14	0.10
Above 4.00	Ohio River above Dam 29	1	7.64	7.46	3.12	3.41	4.50	4.35	0.16	−0.29	0.45
	Ohio River below Dam 29	2	8.17	7.95	3.93	3.47	4.24	4.48	0.22	0.46	−0.24
	Ohio River Cincinnati pool	13	8.28	8.03	3.59	3.64	4.67	4.39	0.25	−0.05	0.30
	All tributaries	57	8.59	8.32	3.69	3.51	4.90	4.81	0.27	0.18	0.09
Average of all in B. O. D. range		73	8.51	8.25	3.67	3.53	4.84	4.72	0.26	0.14	0.12
Average of all samples in entire range		1396	8.41	8.23	6.75	6.63	1.66	1.60	0.18	0.12	0.06

[a] From 6 sampling points in a 55-mile stretch of river.
[b] From 6 sampling points in a 57-mile stretch of river.
[c] From 3 sampling points in a 20.4-mile stretch of river.
[d] From 14 sampling points distributed among 6 tributaries.
[e] From sampling points distributed among 8 tributaries.

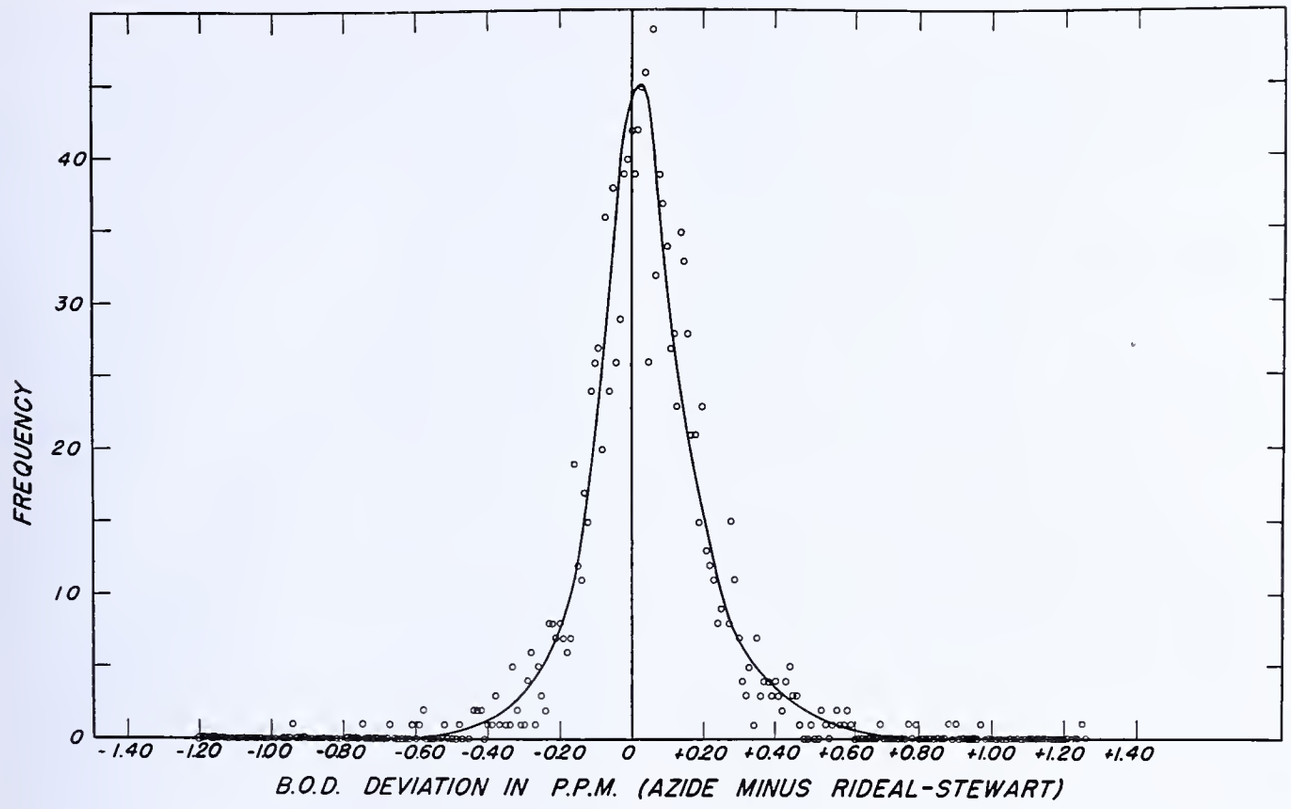

FIGURE 1. FREQUENCY OF DEVIATIONS

amounts of B. O. D. on the comparative results. A study of the mean values obtained shows a deviation varying from 0.04 p. p. m. in the lower range to 0.12 p. p. m. in the highest. Apparently the deviation in 5-day B. O. D. between the two methods is not an absolute deviation, but involves an extremely small portion of the B. O. D. in the higher ranges. However, this discrepancy is so slight, all mean results being within or just outside the experimental limits of error, that it possesses little real significance. An over-all average, involving 1396 samples, reveals a deviation of 0.06 p. p. m. in the 5-day B. O. D.

A frequency curve, Figure 1, confirms the conclusions drawn from the mean results and shows the range of the deviations. The most probable deviation is seen to be 0.05 p. p. m. It is obvious that the azide method and the Rideal-Stewart modification are equally satisfactory in determining the 5-day B. O. D. on samples from streams such as the middle and lower Ohio and its tributaries or for use in comparable work.

Determination of the 5-day B. O. D. by the modified azide and Rideal-Stewart modifications was also studied on the Scioto River. The Scioto, a small stream, carries a proportionately greater pollution load than the Ohio. Above Shadeville, it receives the effluent from the Columbus Sewage Treatment plant. At the Pennsylvania Railroad Bridge (two miles below Circleville) it received, during the period studied, the untreated sewage of the City of Circleville and waste from the strawboard plant located there. Samples collected at Kilgore contain the effluent from the primary treatment plant at Chillicothe and wastes from the two paper mills located there. Stream flow varies widely even over short-time intervals. Nitrites are always found in the stream, varying with the point of collection and the season from a few hundredths to several parts per million.

The Scioto constitutes a more stringent basis of comparison than the Ohio. The mean results, presented in Table II, are similar to those obtained on the Ohio, but of slightly greater magnitude. The greatest deviation occurs at the Pennsylvania Railroad Bridge sampling station. On occasion the 5-day B. O. D. was as high as 30 p. p. m. at this point, necessitating dilution of the samples before incubation. This introduces a new factor. Any difference between the two methods, strictly speaking, is shown only by the deviations in the amounts actually incubated or in the differences between actual titrations. This deviation is then magnified mathematically in calculating the B. O. D. by the dilution factor as follows:

Deviation in B. O. D. =
dilution factor × deviation in actual titration

This is a difficulty inherent in any dilution method. If the mean deviation of 0.29 p. p. m. obtained at the Pennsylvania Railroad Bridge, for example, is recalculated on the basis of actual titrations, it is reduced to 0.18 p. p. m. Similar reductions could be expected at other stations. These results indicate that the azide and the Rideal-Stewart modifications of the Winkler method are in remarkably close agreement in determining the biochemical oxygen demand on river samples.

TABLE II. SUMMARY OF COMPARATIVE STUDY OF AZIDE AND RIDEAL-STEWART METHODS

(Determining 5-day B. O. D. of Scioto River samples)

Sampling Point	Miles from Columbus	Number of Samples	Mean B. O. D. Azide	Mean B. O. D. Rideal-Stewart	Deviations, Azide − Rideal-Stewart
Shadeville	13	30	7.53	7.44	+0.09
Circleville	33	8	2.59	2.52	+0.07
Pennsylvania R. R.	35	21	5.14	4.85	+0.29
Chillicothe	61	30	3.53	3.50	+0.03
Kilgore	67	36	3.21	3.12	+0.09
Higby	76	29	4.57	4.51	+0.06
All samples		154	4.60	4.50	+0.10

Comparison of Preliminary Acid-Azide Treatment with Alsterberg Procedure

When the sodium azide modification for the destruction of nitrites is used, the original technique may be varied by adding the azide as a separate solution in a preliminary step

after acidifying the sample. This method was employed in the foregoing study.

The comparative reliability of these two methods was investigated on samples from the Ohio River and its tributaries in the Cincinnati area.

Table III. Comparison of Preliminary Acid-Azide Treatment with Alsterberg Procedure

(On a series of 184 Ohio River and tributary samples. Mean values)

	Initial Dissolved Oxygen	Final Dissolved Oxygen	5-Day B. O. D.
	P. p. m.	*P. p. m.*	*P. p. m.*
Preliminary acid-azide treatment	7.43	5.47	1.95
Alsterberg procedure	7.42	5.45	1.96

A total of 184 samples were analyzed in duplicate and the results are summarized in Table III. The mean results exhibit a remarkable agreement, the deviation being only 0.01 p. p. m. in the initial dissolved oxygen, 0.02 p. p. m. in the final dissolved oxygen, and 0.01 p. p. m. in the 5-day B. O. D. Evidently from the standpoint of reliability in ordinary work there can be no choice between the two methods. For routine work where difficulties are not likely to be encountered, the Alsterberg procedure must be recommended, combining as it does both shortened time and simplified manipulations. The greater flexibility of the preliminary acid-azide treatment, on the other hand, must be understood in any evaluation of the scope of these two methods. In any case when the period of alkalinization must be shortened or the technique of the so-called "short Winkler" method is employed, the preliminary acid-azide treatment is recommended. It appears also to have merits in delayed titrations.

It has been observed that a 2 per cent sodium azide solution is a very stable reagent. Solutions that have been kept for one year in the authors' laboratory have given results in complete agreement with freshly prepared azide solutions when used on duplicate samples.

Use of Azide to Prevent Oxidation during Storage of Samples

In making dissolved oxygen determinations, it is frequently convenient or expedient to delay the titration. As the azide method showed some promise in this contingency, an investigation was undertaken to demonstrate its value for this purpose.

The materials selected for this experiment were a 10 per cent mixture of sewage in dilution water, the effluent from an experimental trickling filter, and the effluent from an experimental activated sludge unit. This latter effluent was aerated to increase the oxygen content. The results on these samples constitute a more severe test than would ordinarily be encountered in stream pollution studies. Ten series of tests were made on each material, all determinations being made in triplicate. In each series twenty-one bottles were put up. Three of these were dosed completely, using the azide method, and titrated to obtain an initial dissolved oxygen. The remainder were divided into three groups of six each. The first group was dosed completely through the final acidification and stored; the second was treated with acid and azide and stored; the third was dosed with acid only and stored. This storage period, at room temperature, was divided into two intervals, a fairly short one of 2 to 5 hours, designated as A, and a longer one varying from 21 to 29 hours from the start, designated as B. Then, A hours later three samples from each group were taken and the determination was completed when necessary, and titrated. After B hours this procedure was repeated with the remaining samples.

The mean values of the data obtained are presented in Table IV. An inspection indicates the value of the azide in delayed titrations. It is immediately apparent that, even after 5 hours, the samples dosed with azide check very closely with the initials run, these deviations being only 0.04, 0.06, and 0.08 p. p. m. The samples dosed with acid, on the contrary, while showing close agreement on the 10 per cent sewage, show deviations of 0.30 p. p. m. using trickling filter effluent and 0.22 p. p. m. using activated sludge effluent. Complete treatment shows a considerable deviation, 0.22 p. p. m., with the trickling filter effluent, but close agreement on the sewage and the activated sludge effluent. When the data relative to the longer time interval, 21 to 29 hours, are examined, these deviations become more pronounced. Agreement is closest on the 10 per cent sewage samples, the complete treatment and acid giving deviations of 0.18 and 0.15 p. p. m., respectively, as compared with 0.10 p. p. m. for the acid-azide treatment. When trickling filter effluents are used, the deviations are more marked, 0.69 p. p. m. and 0.98 p. p. m., respectively, for the complete treatment and acid dosage, and only 0.25 p. p. m. for the acid-azide treatment. Similarly, with activated sludge effluents these deviations are 0.34 and 0.81 p. p. m. for complete treatment and acid dosage, and 0.26 p. p. m. for acid-azide treatment. These deviations calculated to a percentage basis are given below to emphasize further the general superiority of the acid-azide treatment as a preservative during periods of sample storage preliminary to dissolved oxygen determination:

	Percentage Deviation after Storage for 21 to 29 Hours		
	Complete treatment	Acid treatment	Preliminary acid-azide treatment
10% raw sewage	2.9	2.4	1.6
Trickling filter effluent	15.5	21.9	5.6
Activated sludge effluent	6.0	14.5	4.7

Use of Preliminary Acid-Azide Treatment in Field Work

During the course of a study of the Scioto River, the feasibility of a preliminary acid-azide treatment of dissolved oxygen samples, to be performed by the sample collector, was studied. Duplicate samples were collected, one being dosed with acid and azide in the field and the other being brought to the laboratory undosed. Here the azide determination was completed and the other sample was analyzed by the Rideal-Stewart method. The time in transit averaged about 2.5 hours varying between extremes of 1 and 5 hours. The mean values for dissolved oxygen (p. p. m.) obtained from 63 samples are: preliminary acid-azide treatment in field by Winkler procedure completed in laboratory, 8.44; Rideal-Stewart procedure in laboratory, 8.21; deviation, 0.23.

These results agree very closely with observations previously reported (*6*). The mean deviation of 78 samples

Table IV. Use of Azide to Prevent Oxidation during Storage

	Initial Dissolved Oxygen	Observed Dissolved Oxygen after Interval of Storage			Dissolved Oxygen Loss after Period of Storage by Indicated Treatment		
		After complete azide Winkler treatment	Acid-azide–treated sample	Acid-treated sample	Complete	Acid-azide	Acid
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
			Interval A				
10% raw sewage	6.31	6.25	6.25	6.24	0.06	0.06	0.07
Trickling filter effluent	4.46	4.24	4.42	4.16	0.22	0.04	0.30
Activated sludge effluent	5.58	5.50	5.50	5.36	0.08	0.08	0.22
			Interval B				
10% raw sewage	6.31	6.13	6.21	6.16	0.18	0.10	0.15
Trickling filter effluent	4.46	3.77	4.21	3.48	0.69	0.25	0.98
Activated sludge effluent	5.58	5.24	5.32	4.77	0.34	0.26	0.81

reported at that time, on samples having a dissolved oxygen content over 7.0 p. p. m., was 0.31 p. p. m. These observations were obtained on duplicates dosed in the laboratory. It is apparent that in this particular set of data there is no advantage in a preliminary acid-azide dose performed in the field, probably because the B. O. D. of these samples was very low. With samples in higher B. O. D. ranges this procedure probably would be of value in field work or where samples have to be transported for a considerable distance for laboratory examination.

It has long been known that various substances interfere with the Winkler procedure. Modifications have been devised to overcome these difficulties, one such being the azide modification for the destruction of nitrites, discussed in this paper. These modifications themselves possess limitations and should not be used indiscriminately. Certain examples of interference have been noticed. Some of these have been confirmed; others are indicated by preliminary data and merit further investigation. The azide method is not recommended for the determination of dissolved oxygen in suspensions of river mud (*5*), or in the presence of ferrous and/or ferric iron, or in raw undiluted sewage. Tentatively, pending further investigation, the azide method is not recommended in the presence of considerable amounts of copper or of sulfite wastes or for any industrial waste containing reducing or oxidizing materials.

Summary

The azide and the Rideal-Stewart modifications of the Winkler method are equally efficacious for the determination of B. O. D. in river pollution studies. Either may be expected to give reliable results.

The azide reagent may be added in a preliminary step following acid or it may be added in combination with the alkaline-iodide Winkler reagent. The first method affords greater flexibility, while the latter has manipulative advantages for routine work. The authors' results on samples from a 267-mile stretch of the Ohio River, extending from 265 to 532 river miles below Pittsburgh, and from tributaries in the area, indicate that these methods will be in complete agreement.

A preliminary treatment with acid and azide, whenever it may be necessary or convenient to store a sample for a short time, is advantageous in preventing oxidation. Such a procedure is superior to a complete Winkler treatment through the final acidification, when the titration is delayed.

A preliminary acid-azide treatment will probably be useful in the determination of dissolved oxygen when the samples are to be transported for a considerable distance to a central laboratory.

Acknowledgment

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Determination of Levulose in the Presence of Dextrose and Sucrose. A Ferricyanide Method

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BECAUSE there are only slight differences in the rates of reaction of dextrose and levulose with most oxidizing agents, the determination of one sugar in the presence of the other presents a much more difficult problem than the determination of total reducing sugar.

At the present time, the only chemical method for levulose in general use is Jackson and Mathews' (*3*) modification of the method proposed by Nyns. In this procedure, it is necessary to introduce a factor to correct for the reducing action of the dextrose that is present. Fischl (*2*) described a procedure which uses a reagent composed of cupric sulfate, Rochelle salt, sodium carbonate, and disodium phosphate. He reported that, under the conditions specified, levulose has a reducing action 167 times as great as dextrose, although no analytical results are given to substantiate this. A method developed by Strepkov (*6*) was reported several years ago; the reagent used is a 0.005 *M* solution of potassium ferricyanide containing 80 grams of disodium phosphate per liter. It has been impossible, however, to confirm his finding that dextrose has no reducing action at all under the specified conditions.

A previous publication (*1*) reported the results of a study of the effect of temperature and the concentration of sodium carbonate and disodium phosphate in the ferricyanide reagent upon the rates of oxidation of the two sugars. Further consideration of the reaction has shown that the basis for calculation and expression of the results of the previous paper is somewhat misleading. It was believed that a near-stoichiometric relationship prevailed between the reactants. As a consequence, when ferricyanide was in excess, it was assumed that the quantity of sugar oxidized was proportional to the amount of reagent reduced. The fact that the amount reduced reached a maximum and constant value after the mixture had been heated on a steam cone for 10 minutes caused this value to be assigned as that of 100 per cent oxidation for the quantity of sugar taken. However, as will be shown, the equivalent relationships of the reactants change considerably with change in the composition of the reagent and the conditions of the reaction. Hence, the values in the previous paper for per cent of sugar oxidized are only "apparent" but the results as expressed show a correct relationship of the various effects, since the apparent per cent oxidation is directly proportional to the amount of reagent reduced.

As the previous paper has shown, the reaction was very rapid at boiling temperature, and no great selectivity could be obtained. At 50° C., however, the reaction was slower but showed a much greater difference in the oxidation rates of levulose and dextrose. Since a temperature of 50° C. was

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most selective, it was used in most of this later work. The effect of increasing the concentration of sodium carbonate in the reagent was to increase the rate of oxidation of both sugars. Disodium phosphate specifically decreased the reaction rate for dextrose without affecting that for levulose. The most satisfactory reagent was found to be one which contained high concentrations of these two salts along with the potassium ferricyanide. These characteristic effects have been standardized and adapted to a method for the quantitative estimation of levulose in the presence of dextrose and sucrose.

Experimental

All the inorganic reagents used were of analytical grade, as were also the sucrose and dextrose. The purity of the levulose was determined by polarization and found to be 98.6 per cent. Analysis by the chemical method of Jackson and Mathews checked the purity value indicated by the optical method.

In addition to disodium phosphate and sodium carbonate, a number of other salts were subjected to a preliminary examination in an effort to find some which would be more selective. Sodium acetate, sodium citrate, borax, trisodium phosphate, and disodium arsenate were tried but none proved to be so satisfactory as the two used. Borax decreased the oxidation rate of both sugars but affected levulose to a much greater extent than dextrose. Sodium citrate did not show any valuable properties and is objectionable in the final estimation of ferrous ion, in that citrates are also oxidized by ceric sulfate at room temperature and another reagent must be used at this stage of the procedure. Trisodium phosphate was so alkaline that even small amounts decreased the selectivity of the reagent. Sodium acetate was not alkaline enough to replace sodium carbonate and did not show any specificity for either sugar. Disodium arsenate worked as well as disodium phosphate but showed no particular advantages.

Sodium carbonate and disodium phosphate, then, appear to possess to a high degree the properties which are necessary for the levulose reagent and their use was continued in the final study.

Factors Affecting Levulose-Ferricyanide Equivalence. The effect of variation in the concentration of the salts in changing the equivalence of the sugar oxidized and the ferrocyanide formed was established and the results are shown in Table I. With higher salt concentrations more ferrocyanide is formed in the oxidation of a fixed amount of sugar. The same effect is obtained by increasing the concentration of the potassium ferricyanide.

Table I. Effect of Composition of Reagent upon Levulose-Ferricyanide Equivalence

(5 mg. of levulose heated for 10 minutes at 100° C.)

25 Ml. of Reagent Containing 4 Grams of $K_3Fe(CN)_6$ and Varying Amounts of $Na_2HPO_4.12H_2O$ and Na_2CO_3 per Liter

$Na_2HPO_4.12H_2O$, grams	..	80	80	250	250
Na_2CO_3, grams	24	24	150	24	150
$Ce(SO_4)_2 \rightleftharpoons K_3Fe(CN)_6$ reduced, milliequivalent	0.1450	0.1539	0.1596	0.1598	0.1619
$Ce(SO_4)_2$ per mg. levulose, milliequivalent	0.0289	0.0307	0.0319	0.0320	0.0323

25 Ml. of Reagent Containing 24 Grams of Na_2CO_3 and Varying Amounts of $K_3Fe(CN)_6$ per Liter

$K_3Fe(CN)_6$, grams	4	7	8	10	12	24
$Ce(SO_4)_2 \rightleftharpoons K_3Fe(CN)_6$ reduced, milliequivalent	0.1450	0.1509	0.1518	0.1558	0.1569	0.1658
$Ce(SO_4)_2$, per mg. levulose, milliequivalent	0.0289	0.0302	0.0304	0.0314	0.0316	0.0332

Table II. Standard Levulose Series

(10 ml. of sugar solution heated 60 minutes at 50° C. with 25 ml. of reagent)

Levulose	$Ce(SO_4)_2 \rightleftharpoons K_3Fe(CN)_6$ Reduced	Average Deviation for 28 Determinations
Mg.	*Milliequivalents*	*Milliequivalent*
4.9	0.226	0.001
9.9	0.450	0.002
19.7	0.874	0.002
29.6	1.296	0.003
39.4	1.700	0.002
49.3	2.089	0.002
59.2	2.460	0.007
69.0	2.813	0.007
78.9	3.152	0.008
88.7	3.477	0.008

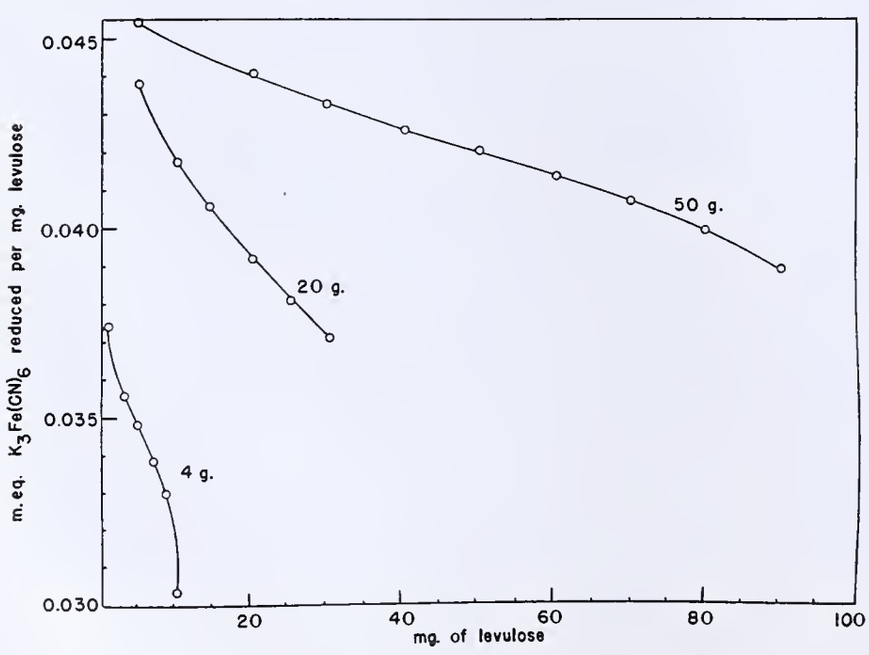

Figure 1. Effect of Ferricyanide Concentration upon Levulose-Ferricyanide Equivalence

Levulose heated at 50° C. for 75 minutes with 25 ml. of reagent containing 80 grams of $Na_2HPO_4.12H_2O$ and 150 grams of Na_2CO_3 in addition to varying amounts of $K_3Fe(CN)_6$

Since the ferricyanide concentration is so important, it was thought that a large excess might improve the equivalent relation of the reactants. The ferricyanide concentration was, therefore, increased from the usual 4 grams per liter to 20 and 50 grams per liter. As Figure 1 shows, the levulose-ferricyanide equivalence was definitely improved and the concentration range for the levulose increased ninefold.

The high concentration of ferricyanide gave rise to two difficulties. 1,10-Phenanthroline, which had been used as the indicator in all the previous work, could not be used because it formed an orange colloidal suspension which gave no color change at the end point. However, a 0.005 *M* solution of sodium diphenylamine sulfonate, prepared according to the directions of Sarver and Kolthoff (*5*), worked very satisfactorily and gave a good color change at the end point. The second difficulty was the formation of a light blue-green precipitate which proved to be cerous phosphate. At times, the precipitate was so heavy that it partially masked the end point, particularly if the titration was not carried out with sufficient rapidity. It was possible, by increasing the volume of the solution in the flask and carrying out the titration rapidly, to reduce greatly the amount of precipitate formed so that it did not interfere with the end point.

The reagent finally chosen contained 50 grams of potassium ferricyanide, 225 grams of disodium phosphate dodecahydrate, and 150 grams of anhydrous sodium carbonate per liter. This reagent was stored in a black bottle and all light was excluded. Under these conditions, there was an initial aging period of 6 to 8 days during which the reagent changed by about 2 per cent, but it remained perfectly stable during the next 30 days, which was the longest period over which the tests were conducted. A yellow precipitate formed in the solution on standing. This precipitate may be filtered off, or the delivery tube kept several centimeters above the bottom of the bottle. Even large amounts of this precipitate do not, however, interfere with the determination.

Conditions for Oxidation Procedure. The specific conditions for carrying out the

oxidation were studied in order to keep the error below a definite limit.

If the ferricyanide reagent stands in contact with the sugar solution at room temperature for any appreciable length of time previous to heating, sufficient oxidation occurs to cause a serious error in the determination. To avoid this effect, the reagent must be added to the sugar solution just before the flask is placed in the bath.

In order that duplicate levulose estimations shall not differ more than 0.1 mg., the temperature must be maintained at 50 ± 0.05° C. and the heating must be carried out for 60 minutes ± 5 seconds. The 60-minute period was chosen because, as shown in Figure 2, the curve levels off rapidly at this point and it is not an inconveniently long time.

The volume of sugar solution must be exactly 10 ml. and the 25-ml. portion of the ferricyanide reagent must be within ±0.1 ml. of this volume.

The reaction vessel used was a 125-ml. Erlenmeyer flask, but larger sizes can be used without affecting the results to an appreciable extent. A strip of sheet lead made into a collar is fitted over the flask to prevent its tipping or floating in the water bath. The water level in the bath should extend 1 or 2 cm. above the level of the solution in the flask.

After the 60-minute heating period, the flask is removed from the bath and immediately cooled in cold water, and the solution is carefully acidified with 60 ml. of 3 *N* sulfuric acid. The acid must be added slowly and the flask shaken continuously to prevent spattering by the sudden evolution of carbon dioxide[1]. The indicator, 6 to 8 drops of 0.005 *M* sodium diphenylamine sulfonate, is added and solution titrated to the appearance of a red color with a standard ceric sulfate solution which is 0.1 *N* to 0.15 *N*.

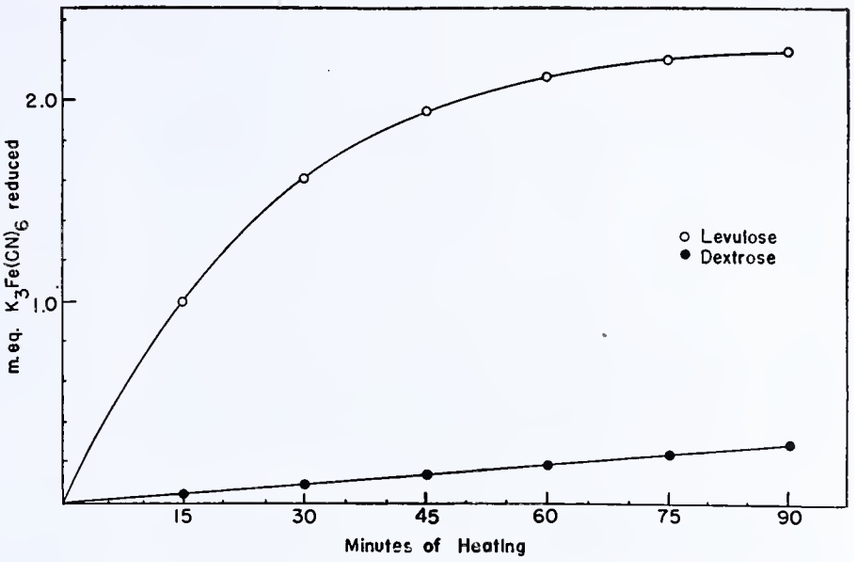

FIGURE 2. RATE OF OXIDATION OF LEVULOSE AND DEXTROSE

50 mg. of sugar heated at 50° C. with 25 ml. of reagent containing 50 grams of $K_3Fe(CN)_6$, 225 grams of $Na_2HPO_4.12H_2O$, and 150 grams of Na_2CO_3

STANDARD LEVULOSE SERIES. A standard levulose series was made and all the above-mentioned precautions were observed. The results in Table II are the average of 14 separate determinations made in duplicate with three different batches of reagent over a period of 30 days. These results can be used most readily by plotting a graph on such a scale that levulose can be estimated accurately to 0.1 mg. and ceric sulfate to 0.001 milliequivalent. The weight of levulose used is corrected to 100 per cent purity.

EFFECT OF DEXTROSE UPON APPARENT LEVULOSE VALUES IN A MIXTURE OF TWO SUGARS. The reducing action of dextrose was investigated by using principles employed by Jackson and Mathews (*3*). Weighed amounts of mixtures of levulose and dextrose were dissolved in 10 ml. of water and oxidized according to the procedure previously described. The difference between the amount of levulose actually taken and the apparent levulose indicated by the milliequivalents of ceric sulfate consumed represented the reducing action of the dextrose present. The amount of dextrose taken divided by the difference in the two levulose values gave a factor which represented the number of milligrams of dextrose having the same reducing action as 1 mg. of levulose. Column 6 of Table III gives the factors thus obtained for different amounts and proportions of levulose and dextrose.

TABLE III. REDUCING ACTION OF DEXTROSE IN PRESENCE OF LEVULOSE

Ratio of Levulose to Total Sugar	Levulose Taken	Dextrose Taken	$Ce(SO_4)_2$ Used	Apparent Levulose Found	Dextrose Equivalent to 1 Mg. of Levulose	Dextrose Taken Divided by Factor[a]	Corrected Levulose	Error in Levulose Found
%	*Mg.*	*Mg.*	*M. eq.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	%
Range $\frac{\text{Levulose}}{\text{Total Sugar}}$, 38 to 56 Per Cent								
50	29.6	30.0	1.380	31.6	15.0	2.0	29.6	0.0
40	38.4	56.7	1.820	42.5	13.8	3.9	38.6	0.5
38	45.8	73.8	2.134	50.5	15.7	4.6	45.9	0.2
56	48.2	38.6	2.140	50.7	15.4	2.4	48.3	0.2
53	53.9	47.6	2.386	57.1	14.9	3.0	54.1	0.4
53	58.3	50.9	2.542	61.4	16.4	3.1	58.1	− 0.3
50	69.0	70.0	2.956	73.1	17.1	4.3	68.8	− 0.3
Range $\frac{\text{Levulose}}{\text{Total Sugar}}$, 30 to 36 Per Cent								
30	9.9	20.0	0.508	11.2	15.4	1.4	9.8	− 1.0
32	25.2	52.9	1.254	28.6	15.6	3.6	25.0	− 0.8
33	29.6	60.0	1.454	33.4	15.8	4.1	29.3	− 1.0
33	49.3	100.0	2.313	55.2	16.9	6.2	49.0	− 0.6
33	56.6	113.0	2.623	63.5	16.4	7.0	56.5	− 0.2
36	66.6	116.3	2.985	73.9	15.9	7.2	66.7	0.1
36	67.2	118.7	3.009	74.6	16.1	7.4	67.2	0.0
33	69.0	140.0	3.100	77.4	16.7	8.7	68.7	− 0.4
32	76.6	162.1	3.410	86.7	16.1	10.1	76.6	0.0
Range $\frac{\text{Levulose}}{\text{Total Sugar}}$, 23 to 28 Per Cent								
25	22.9	69.0	1.221	27.8	14.1	4.7	23.1	0.9
23	29.6	100.0	1.577	36.4	14.7	6.9	29.5	− 0.3
24	38.1	117.0	1.957	45.9	15.1	8.1	37.8	− 0.8
28	43.4	112.6	2.153	51.0	14.8	7.0	44.0	1.4
23	46.1	151.0	2.345	56.0	15.3	9.4	46.6	1.1
25	49.3	150.0	2.428	58.3	16.7	9.3	49.0	− 0.6
25	69.0	210.0	3.261	82.2	15.9	13.0	69.2	0.3
78	87.3	24.8	3.474	88.7	17.7	1.5	87.2	− 0.1
							Av. (of 24)	0.5
Range $\frac{\text{Levulose}}{\text{Total Sugar}}$, Less than 20 Per Cent								
5	38.4	670.0	3.162	79.2	16.4	45.9	33.3	−13.3
9	24.4	237.0	1.741	40.5	14.7	16.2	24.3	− 0.4
12	7.3	54.8	0.523	11.5	13.1	3.8	7.7	5.5
15	9.9	50.0	0.609	13.5	13.9	3.4	10.1	2.0
16	14.8	80.6	0.924	20.8	13.4	5.5	15.3	3.4
17	20.1	91.6	1.174	26.7	13.9	6.3	20.4	1.5
							Av.	4.4

[a] 14.6 with less than 40 mg. of levulose, 16.1 with more than 40 mg. of levulose.

[1] Since the completion of this paper, W. J. Shannon, who has been using the method, has made the following suggestions as improvements: Increase the size of the reaction flask to 200 ml. and introduce the sulfuric acid from a separatory funnel or other suitable device with a glass stopcock, so that the flow may be regulated as the carbonate is neutralized and the danger of loss of solution minimized. Use ice in cooling the flask after the heating period.

Table IV. Effect of Sucrose upon Levulose-Dextrose Reducing Ratio

Levulose Taken	Dextrose Taken	Sucrose Taken	$Ce(SO_4)_2$ Used	Apparent Levulose Found	Dextrose Taken Divided by Factor[a]	Corrected Levulose	Error in Levulose Found
Mg.	*Mg.*	*Mg.*	*M. eq.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0	0	210.0	0.002	0.05	...	0.05	0.05
0	0	700.0	0.006	0.13	...	0.13	0.13
0	100.0	210.0	0.349	7.5	6.9	0.6	0.6
							%
49.3	100.0	210.0	2.317	55.3	6.2	49.1	−0.4
49.3	0	210.0	2.082	49.2	...	49.2	−0.2
45.1	148.6	970.7	2.264	53.9	9.2	44.7	−0.9
51.5	0	1720.0	2.144	50.8	...	50.8	−1.4
22.0	102.7	552.0	1.267	28.9	7.0	21.9	−0.5
7.1	161.3	581.1	0.828	18.5	11.1	7.4	4.2
41.4	70.4	310.5	1.960	46.0	4.4	41.6	0.5
42.0	72.4	992.4	1.980	46.5	4.5	42.0	0.0

[a] 14.6 for less than 40 mg., 16.1 for more than 40 mg. of levulose.

In general, this factor was not constant and tended to increase slightly with increasing amounts of levulose. To ensure greater accuracy, two factors were used for different amounts of levulose. When less than 40 mg. of levulose was present, the average factor was 14.6; and when more than 40 mg. were present, the factor was 16.1. The amount of dextrose actually taken was then divided by the proper factor, determined by the amount of levulose present, to give a subtractive correction which was applied to the apparent levulose value to obtain the true amount of levulose.

The accuracy of the determination depends, not so much on the amount of levulose present, as on the ratio of levulose to dextrose. When the levulose present was more than 20 per cent of the total reducing sugar, the average error was 0.5 per cent; but when levulose represented less than 20 per cent of the reducing sugar, the error became much greater. This is to be expected, since the correction becomes large as compared to the amount of levulose present.

Sucrose Effect. The effect of this reagent on sucrose under the specified conditions was studied. When no other sugars were present, 700 mg. of sucrose had the same reducing action as 0.13 mg. of levulose. When mixtures of the three sugars were analyzed, levulose was determined with about the same accuracy as in the absence of sucrose. Sucrose may be present in amounts of 10 to 20 times that of the total reducing sugar without causing any appreciable error in the levulose determination. Some of the results obtained with sucrose present are given in Table IV.

Procedure and Calculation for Unknown Sample. Exactly 10 ml. of the sugar solution, which contains not more than 90 mg. of levulose, or its equivalent in a levulose-dextrose mixture, are treated with 25 ± 0.1 ml. of ferricyanide reagent and heated at 50 ± 0.05° C. for 60 minutes ± 5 seconds. The flask is removed from the bath, immediately cooled in cold water, and carefully acidified with 60 ml. of 3 *N* sulfuric acid, 6 to 8 drops of 0.005 *M* sodium diphenylamine sulfonate indicator solution are added, and the ferrocyanide is titrated with a 0.1 *N* to 0.15 *N* standard ceric sulfate solution.

The milliequivalents of ceric sulfate required are referred to the graph plotted from the figures in Table II to obtain the apparent levulose value. The apparent levulose is subtracted from the total reducing sugar present, which has been previously determined by any standard method, such as that of Lane and Eynon (*4*), to give a figure for the apparent dextrose present. This apparent dextrose value divided by the proper factor gives a correction which is subtracted from the apparent levulose to give a new approximation. This new levulose value subtracted from the total reducing sugar gives a new dextrose value, which is again divided by the factor to give a second correction which is applied to the original apparent levulose. This calculation is repeated until successive values remain constant. Two, or sometimes three, approximations are sufficient to obtain constant values. In choosing the proper factor, approximately one fifteenth of the apparent dextrose must be subtracted from the apparent levulose figure to obtain an estimation of the actual levulose value, if the levulose present is close to 40 mg., or there is a large amount of dextrose.

Discussion

This procedure is, like most sugar methods, an empirical one and, consequently, any deviation from the standard procedure will cause an error in the results. The time and temperature of heating must be adhered to very closely.

If the reagent is stored away from all light, it is stable for at least 30 days after an aging period of 1 week. It is recommended that the reagent be tested frequently against pure levulose if it is kept for more than 30 days.

If maltose or lactose is present, the method is not applicable since they both exert a reducing action of about the same magnitude as does dextrose. However, a rough approximation of the amount of levulose can be obtained by considering all the other reducing sugars to be dextrose and making the calculation on this basis.

The formation of cerous phosphate precipitate is an objectionable feature. However, if the titration is carried out as rapidly as possible after it has been started, the interference is small. Another disadvantage is the use of two factors in the calculation of the amount of levulose, but it is felt that the increased accuracy more than compensates for the trouble involved. The chief advantage of the method lies in the direct procedure for finding the amount of ferrocyanide formed. If a water bath is used, which will hold 12 flasks at the same time, one operator can complete 18 determinations in duplicate during a 4-hour period. This is done by removing flasks from the bath at 5-minute intervals, which give sufficient time for carrying out the titration and preparing the next sample.

Summary

A method is described for the determination of levulose in the presence of dextrose and sucrose. The reagent used contains 50 grams of potassium ferricyanide, 225 grams of disodium phosphate dodecahydrate, and 150 grams of anhydrous sodium carbonate per liter. The oxidation is made at 50° C. for 60 minutes. A maximum of 90 mg. of levulose, or its equivalent in a levulose-dextrose mixture, can be determined.

Dextrose exerts a small, but definite, reducing action and a factor is introduced to correct for its presence. Sucrose has very little effect on the determination and can be present in large quantities without interfering appreciably.

When the levulose present is 20 per cent or more of the total reducing sugar, an average accuracy of 0.5 per cent can be obtained. The error increases rapidly as the ratio of levulose to total reducing decreases below this value.

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From a portion of the doctorate thesis of H. C. Becker, 1940.

Rapid Detection of Chromium

In Stainless Steels, Other High-Chromium Alloys, and Plating

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CHROMIUM in high-chromium alloys and plating may be detected rapidly by excellent chemical methods, utilizing the *s*-diphenylcarbazide reaction with chromate ion (*2–4, 7*). The rapidity of the tests is limited, however, by the time required for solution in acids, oxidation to chromate ion, and the removal of interfering ions.

An expeditious electrographic test for chromium plating is described by Arnold (*1*) and by Glazunov and Křivolahvý (*5*), in which the chromium is dissolved, anodically, as chromic acid by means of an electric current. A characteristic spot is formed on filter paper moistened with a solution of an electrolyte and benzidine or *s*-diphenylcarbazide, at the surface adjacent to the plating where the current enters the paper. The test has been applied to the detection of chromium in steel and cast iron (*6, 10*).

Two electrographic tests for the detection of chromium in high-chromium alloys and in plating, employing a characteristic reaction of chromium—namely, the formation of blue perchromic acid—have been developed by the author. Elements, such as molybdenum, manganese, and mercury, which interfere with the *s*-diphenylcarbazide test, do not produce interfering colors. The tests may be performed in approximately one second, with little effect upon the surface examined. One of the tests responds to chromium in plating only. The second test responds to chromium in high alloys and in plating. Since the initial sensitivity of the latter test occurs near the minimum chromium content of the stainless steel group, it is particularly useful in the identification of high-ferrous alloys.

ANODIC SOLUTION OF ACTIVE AND PASSIVE CHROMIUM ALLOYS AND PLATING. Polyvalent elements in the active state always dissolve anodically to form ions of the lowest valence, while anodes of the polyvalent elements which become passive, enter solution as ions of a higher valence than when in the active state. Active chromium yields Cr^{++} upon anodic solution, while passive chromium yields CrO_4^{--} (*8, 9*). The author has found that the chromium in active and passive high-chromium iron and nickel alloys and in plating behaves in the same manner and that the high-chromium alloys can be made active or passive by the influence of chloride or nitrate ions, respectively. Chromium in plating remains passive in the presence of either anion, under the conditions of the test, and dissolves as CrO_4^{--}. The iron and chromium components of the stainless steels in the active state enter solution as Fe^{++} and Cr^{++} and, in the passive state, as Fe^{+++} and CrO_4^{--}. An e. m. f. of 6 to 9 volts, a current density of 0.5 to 1.5 amperes per sq. cm. of anode, and 30 per cent solutions of sodium chloride and sodium nitrate have been employed in the tests.

FORMATION OF BLUE PERCHROMIC ACID. When chromate ion enters a solution containing hydrogen peroxide, it reacts with the peroxide ion to form blue perchromic acid in the acidic medium adjacent to the anode. By replacing the solution with filter paper moistened with the solution, the sensitivity of the test is increased as the diffusion of the perchromic acid is retarded. The wad of moistened filter paper is placed between the anode and the cathode in such a manner as to permit the flow of electric current through the paper. When chromate ion is formed upon solution of the anode, a purple-blue spot is found on the side of the paper adjacent to the anode. The spot begins to fade, after a few seconds, as the perchromic acid commences to decompose. The initial intensity and the duration of the spot increase with the chromium content of the specimen.

Apparatus and Test Solutions

The apparatus consists of six large 1.5-volt dry-cell batteries of the bell-ringing type connected in series, a cylindrical graphite rod about 7.5 cm. (3 inches) long and 0.6 cm. (0.25 inch) in diameter, two lengths of insulated flexible copper wire with suitable connectors, and "ashless" quantitative filter paper, 7.5 to 10 cm. (3 to 4 inches) in diameter. The graphite rod is made the negative electrode and the test metal, the anode. Filter paper moistened with solution, placed between the graphite rod and the test metal anode, completes the electric circuit.

TEST SOLUTION A (for chromium plating). Dissolve 30 grams of reagent grade sodium chloride in sufficient 3 per cent hydrogen peroxide solution, U. S. P., to make 100 ml. and filter.

TEST SOLUTION B (for chromium in plating and high-chromium alloys). Dissolve 30 grams of sodium nitrate, reagent grade, in a quantity of 3 per cent hydrogen peroxide solution, U. S. P., sufficient to make 100 ml. and filter.

The test solutions should be slightly acid. Hydrogen peroxide solution, U. S. P., usually contains enough mineral acid to make a suitable test solution; 0.8 ml. of 1 *N* nitric or hydrochloric acid should be added if a neutral hydrogen peroxide solution is used in preparing the reagent.

The test solutions made in 100-ml. quantities have remained useful for periods of more than one month. Solutions older than 2 weeks, however, should be checked frequently. Test solution A is the least stable.

Method

A sheet of filter paper is folded in half and the folding is continued in the same direction until a wad, approximately 0.175 cm. (0.7 inch) wide and having a length equal to the diameter of the paper, is made. One end of the wad is dipped into one of the test solutions and the moistened section is applied to the bright surface of the metal specimen (anode). A momentary contact is made with the graphite cathode to the free side of the moistened end of the paper wad. The paper adjacent to the metal surface is examined. A purple-blue spot obtained with test solution B indicates that chromium is present as plating or as a component of a high alloy. If the purple-blue perchromate spot is obtained with test solution A, the chromium is present as plating only. An e. m. f. of 5 to 6 volts has been found to be the most suitable when testing for plating and an e. m. f. of 7.5 to 9, when testing for alloyed chromium.

A firm contact is made with the graphite electrode, lasting from 0.5 to 1 second. The pressure and duration of the contact are easily determined by experimenting with a specimen of stainless steel or chromium plating. The technique is acquired in a few minutes and no further reference to known metals need be made. If the blue perchromate color appears as a ring, too long a contact is being made or too high a voltage is being employed. The color of the spot is best observed in daylight, since the light from an ordinary incandescent lamp is relatively deficient in the blue end of the spectrum. After a few seconds the spot begins to fade, since the perchromic acid is unstable, but the time of duration is more than sufficient for purposes of identification. The initial temperature of the solution and the anode should preferably be below 30° C., since higher temperatures cause a more rapid decomposition of the perchromic acid and, hence, a more weakly colored spot.

Discussion

A positive test for chromium has been obtained using test solution B with ferrous, nickel, and cobalt-tungsten alloys containing more than 12 per cent chromium and with chromium plating. A series of ferrous alloys ranging from 0.6 to 26.5 per cent chromium which have been tested with test solution B has shown that the point of initial sensitivity of the test lies between 9.75 and 12.38 per cent chromium and is probably closer to the lower value.

Test solution A has given a positive test with chromium metal and chromium plating and a negative test with the stainless steels, stainless irons, and the chromium-nickel alloys. A chromium-cobalt-tungsten alloy with a chromium content of 25 to 31 per cent is the only alloy which has given a positive result with both test solutions, the reason being that this alloy remains passive with either test solution and the chromium dissolves as CrO_4^{--}.

Numerous other metals and alloys have been tested with both solutions, and negative results have been obtained in each case: nickel-silver, solders, brasses, white metals, 15 per cent silicon steel, bronzes, copper-nickel alloys, carbon steels, nickel, copper, manganese, molybdenum, tantalum, tungsten, mercury, cadmium, aluminum, tin, zinc, vanadium, silver, and lead. Vanadium gives a red spot with both solutions and silver yields a black spot with test solution B.

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Determination of Cobalt as Trioxalatocobaltiate

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IN CONNECTION with the work on complex compounds in this laboratory the authors have developed a new spectrophotometric method of determining cobalt which is much more rapid than the gravimetric or electrolytic procedures. Numerous colorimetric methods are available (*3*), but certain of these require time-consuming separations, others are applicable only to minute amounts of cobalt, and others have only fair accuracy. The basis of this procedure is the measurement of the absorption at 605 mμ due to the trioxalatocobaltiate ion, $Co(C_2O_4)_3^{---}$, which is produced by oxidation of a cobaltous solution by lead dioxide in a weakly acid solution of potassium oxalate (*4*), according to the reaction

$$2CoSO_4 + 4HC_2H_3O_2 + 7K_2C_2O_4 + PbO_2 \rightarrow 2K_3Co(C_2O_4)_3 + PbC_2O_4 + 4KC_2H_3O_2 + 2K_2SO_4 + 2H_2O$$

A small amount of lead remains in solution, presumably as acetate. The trioxalatocobaltiate anion produced has a dark emerald-green color with a maximum absorption at 605 mμ (*5*). The intensity of the absorption is used as a measure of the cobalt content.

A Bausch & Lomb visual spectrophotometer was used for estimating the absorption, and with a maximum cell length of 5 cm. the solutions actually measured may contain from 2 to 50 mg. of cobalt per 100 ml. Since the volume of the final solution need not exceed 50 ml., the method may be applied to any sample or aliquot containing as much as 1 mg. of cobalt. Nickel, iron, and chromium may be present in considerable amounts; copper and manganese must be previously removed, if present. Although the authors have used a spectrophotometric procedure requiring no standard solutions for comparison, the same method could be adapted for use with a photoelectric colorimeter with a suitable red filter. The analysis can be completed in about 15 minutes from the time the aliquot of cobaltous salt solution is measured out. The accuracy is better than 2 per cent of the quantity determined.

It is necessary to have a standard solution of the pure salt to determine the extinction coefficient at the wave length selected for measurement of the absorption. The preparation of a standard solution of potassium trioxalatocobaltiate is difficult, however, since the water content of the crystalline salt is very variable. Furthermore, the complex is not entirely stable, so that its purity needs to be established. When the salt is prepared by a modification of the procedure of Jaeger and Thomas (*2*) its formula approximates closely to $K_3Co(C_2O_4)_3.3.5H_2O$, but the water content varies with the atmospheric humidity from day to day. Vranek (*5*) measured the absorption of trioxalatocobaltiate solutions. He standardized his solutions by electrolytic determination of total cobalt, however, which of course would give an erroneous extinction coefficient if the salt had decomposed to a cobaltous compound to an appreciable extent. Since he gave no statement as to the history of his material, the authors redetermined the absorption at 605 mμ with solutions standardized by a procedure which they developed for estimating the complex cobaltic ion in the presence of cobaltous ions.

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Volumetric Determination of Trivalent-Cobalt Complex

In acid solution, potassium iodide does not reduce the trioxalatocobaltiate ion rapidly; ferrous sulfate reacts much more readily, but the formation of the greenish-yellow trioxalatoferriate ion in the solution obscures the end point. The authors found that ferrous sulfate may be used in an electrometric titration of the cobaltic ion, though the electrode is very sluggish until the end point is passed. The potential changes by about 150 millivolts at the end point. In the direct titration with Mohr's salt the end point may be seen much more clearly if potassium fluoride is added near the end, but this is objectionable. Even so, the end point is reached slowly at room temperature, and the authors therefore abandoned the attempt to make a direct titration of trivalent cobalt.

The procedure finally developed consists in titrating the cobaltic solution with approximately 0.05 *N* Mohr's salt (20 grams per liter in 2 per cent sulfuric acid) at room temperature until the change from green to yellow shows that the end point has been slightly overstepped. The excess of ferrous ion is then titrated with standard potassium dichromate, using diphenylamine as the indicator. The back-titration of the excess ferrous salt is complicated by the induced reaction between dichromate and oxalic acid, however, so that it is necessary to determine an empirical factor for the dichromate solution in each analysis.

The solution of the trioxalato salt to be analyzed is prepared so as to be nominally about 0.05 *M*. To an aliquot (10 to 25 ml.), add from a buret standard ferrous sulfate until about 0.5 ml. in excess is present. Let the mixture stand for 5 minutes, when the color change should be complete. Then add 1 ml. of a solution containing 10 ml. of phosphoric acid and 25 ml. of sulfuric acid per 100 ml., and dilute to about 75 ml. Add 3 drops of diphenylamine solution (1 gram per 100 ml. of sulfuric acid) and titrate with standard 0.05 *N* potassium dichromate. Not over 1.0 ml.

should be used. Read the two burets, and add to the mixture as much ferrous sulfate as is estimated to be about equivalent to the dichromate consumed in the back-titration. Again titrate with the dichromate. The ratio between the ferrous sulfate and dichromate solutions under the conditions prevailing near the end point is calculated from the supplementary titration; this factor is used to calculate the excess of ferrous salt found in the back-titration. The normality of the ferrous solution in its reduction of the trivalent cobalt may be determined by any of the usual methods.

The following data show the concordance obtained in the titration of equal samples of potassium trioxalatocobaltiate:

	$FeSO_4$ *Ml.*	$K_2Cr_2O_7$ *Ml.*	$FeSO_4$ Required *Ml.*
Sample 1	10.26	0.15	
	0.16	0.21	10.15
Sample 2	10.68	0.56	
	0.38	0.44	10.20
Sample 3	10.72	0.61	
	0.56	0.61	10.16
			Av. 10.17

$FeSO_4$ 0.04950 *N*:10.17 ml. = 0.0297 gram of Co^{+++}

In a comparison of the electrometric titration with the procedure just described the concordance was equally good. Unfortunately, there is no completely stable cobaltic complex which is readily reducible by ferrous sulfate, so that the method cannot be tested upon a pure compound of known composition. The data above correspond to 11.81 per cent of trivalent cobalt in the sample; the theoretical content in the pure salt with 3.5 moles of water is 11.71 per cent, and with 3 moles, 11.93 per cent.

Maximum Extinction Coefficient

For the measurement of the extinction coefficient at 605 mμ, solutions of a recrystallized sample were made up and titrated by the procedure given above. Cartledge and Djang (*1*) have previously found that solutions of potassium trioxalatocobaltiate obey Beer's law in the concentration range suitable for measurement. Small amounts of the purplish-red oxalatocobaltoate resulting from decomposition have no appreciable effect on the absorption, since the extinction coefficient of this ion at 605 mμ is only 1.71, according to Vranek. The authors' extinction curve for the trioxalatocobaltiate was in substantial agreement with Vranek's between 570 and 605 mμ, but the authors found a slightly higher extinction coefficient at the maximum—namely, 162, instead of 161.1—the difference is probably not significant. To calculate the molar concentration, *c*, of the trioxalatocobaltiate ion we therefore have

$$c = \frac{d}{162\,l}$$

in which *d* is the measured density $\left(\log \frac{I_0}{I}\right)$ and *l* is the cell length in cm. If the oxidized solution is diluted to 100 ml. before measurement of the absorption, the weight of cobalt in the sample is given by the relation

$$\text{Mg. of Co} = 36.4\ d/l$$

Spectrophotometric Determination of Cobalt

For the determination of cobalt the authors have ascertained the conditions for the complete conversion of cobaltous salts into the trioxalato cobaltic complex. The solution may contain sulfates, nitrates, or chlorides.

Neutralize an aliquot containing 1 to 50 mg. of cobalt with sodium hydroxide to the appearance of a slight permanent precipitate. The solution at this stage should not exceed 25 ml. Next add 2 ml. of glacial acetic acid, 5 ml. of 20 per cent ammonium acetate, 10 ml. of 1 *M* potassium oxalate, and about 1 gram of manganese-free lead dioxide. Set the mixture aside at room temperature for 5 to 10 minutes away from bright light, and shake at intervals. After not over 10 minutes dilute the unfiltered mixture to either 50 or 100 ml., according to the amount of cobalt present. This can be readily judged by the depth of the color of the oxalatocobaltoate before addition of the lead dioxide. Promptly after dilution filter the solution without suction through a good filter paper, such as Whatman No. 42. Discard the first portion of the filtrate and catch a sufficient volume directly in the absorption cell.

It is essential that there be no turbidity. If it is necessary to filter a second time, an additional portion of lead dioxide is previously added. This is particularly necessary when the cobalt content is above 25 mg. For the measurement, the authors have used cell lengths of 1 to 5 cm. In the comparison cell they use a blank carried through the regular procedure.

Table I. Determination of Cobalt

Co Present *Mg.*	Volume *Ml.*	Cell Length *Cm.*	Density	Co Found *Mg.*	Error *Mg.*
1.56	50	4	0.350	1.59	+0.03
3.89	50	4	0.848	3.86	−0.03
7.78	100	4	0.857	7.80	+0.02
11.75	100	3	0.975	11.82	+0.07
11.75	100	3	0.960	11.64	−0.11
11.94	100	3	0.987	11.97	+0.03
15.56	100	2	0.852	15.5	−0.1
29.5	100	1	0.815	29.65	+0.15
31.1	100	1	0.845	30.8	−0.3
41.45	100	1	1.13	41.1	−0.35

Table I shows the results on a series of samples prepared from standardized solutions of cobaltous sulfate or cobaltous chloride.

Interfering Elements

An advantage of the method lies in the fact that substances having no appreciable absorption in the red do not interfere. Trivalent chromium interferes, but may be oxidized to the weakly absorbing dichromate by preliminary treatment with lead dioxide and nitric acid. In the oxalate mixture ferric and nickel ions have a weak absorption at 605 mμ. To determine the degree of interference the authors have measured their absorption approximately; the results in Table II are expressed in terms of the number of milligrams of cobalt represented by the observed absorption of varying amounts of these elements. The volume of the measured solution was 100 ml. in each case, and the absorption was too weak to be measured accurately in a 5-cm. cell.

Table II. Cobalt Equivalent of Interfering Elements

	Metal *Mg.*	Co Represented *Mg.*
Ni as $Ni(NO_3)_2$	20	0.4
	40	0.7
	100	1.7
Fe as $FeSO_4$	20	0.2
	40	0.2
	100	0.4
Cr as $K_2Cr_2O_7$	20	0.1
	40	0.2
	100	0.3

In the oxalate mixture copper has a strong absorption in the red and must be removed before oxidation of the cobalt. Manganese, if not previously removed, would be oxidized to the trioxalatomanganiate ion. This complex is unstable at room temperature, and its decomposition induces the reduction of the cobaltiate complex, making a determination impossible.

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Segregation of High- and Low-Titer Fatty Acids

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A routine method for the segregation of the high- and low-titer components of mixed fatty acids is described, which requires approximately one hour and separates the two fractions with at least 95 per cent accuracy. A second separation may increase this to 99 per cent or better.

The method may be applied to fats themselves, giving an indication of the distribution of the acid molecules on the glycerol residue, and permitting identification of the free fatty acids present in a fat.

VARIOUS methods have been described by which mixtures of fatty acids may be separated into relatively saturated and unsaturated fractions. Many of these depend upon fractional crystallization, as of the lead soaps (*3*), lithium soaps (*1*), and barium soaps (*7*). Fractional crystallization of the fatty acids themselves has been used in many instances—for example, by Brown and Stoner (*1*) and by Hartsuch (*2*) for the preparation of pure oleic acid. High-titer modifications of unsaturated acids may be separated from their low-titer isomers by similar crystallizations, as mentioned by Ku (*5*) for eleostearic acid and by Kass and Burr (*4*) for linolelaidic acid.

Purification by fractional crystallization is a standard tool, and by the proper choice of solvents, concentrations, and temperatures a wide variety of separations can be made. The purpose of the present work was to study the separations of fatty acids which may be effected in the course of routine analysis by the use of one solvent, one concentration, and one temperature range.

The method described below permits a rapid segregation of high-titer from low-titer acids, as shown in Table I. The time needed for the separation of any acids in the left-hand column from any acids in the right-hand column is approximately one hour. The completeness of separation is shown by a negative Liebermann-Storch test (*6*) on stearic acid segregated from rosin, and by the fact that the iodine value of the high-titer fraction obtained from a segregation of stearic and oleic acids was 8 to 10. A similar separation by the lead soap method may give an iodine value on the "saturated" acids as high as 32 (*3*). The method may be applied to the fats themselves, as well as to their separated fatty acids.

Table I. Segregation of Fatty Acids

High-Titer Fraction	Low-Titer Fraction
1. Saturated fatty acids from C_{12} to C_{20} (lower members of series not studied)	Unsaturated fatty acids related to
2. Waxy acids from degras	1. Oleic
3. Elaidic acid	2. Linoleic
	3. Linolenic
	4. Rosin acids (abietic)
	5. Naphthenic acids
	6. Dichlorostearic acid
	7. Phenylstearic acid

Description of Method

The fatty acids may be obtained by any standard method appropriate to the sample. In the majority of the authors' work, the method used is that given under A. S. T. M. Designation D128-37. The diethyl ether used is removed by blowing with carbon dioxide.

From 1 to 2 grams (weighed to 1 mg.) of the fatty acids are weighed into a 300-ml. Erlenmeyer flask, and 100 ml. of petroleum ether (30° to 80° C.) are added. The acids are dissolved in the petroleum ether by gentle warming and swirling. If the fatty acids contain some material, such as oxidized or hydroxylated fatty acids, which is insoluble in petroleum ether (fraction 1), this is removed by filtration through paper at room temperature. The filtrate and petroleum ether washings are caught in another 300-ml. Erlenmeyer flask, which is immersed in acetone contained in a beaker. Solid carbon dioxide is added to the acetone, the flask being removed from the acetone and swirled occasionally during this chilling. When the temperature of the filter and of the contents of the flask reaches −45° to −50° C., the contents are filtered.

The filter consists of a jacketed funnel, whose construction is shown in Figure 1. The cork is set into the metal funnel with litharge-glycerol cement. The outer funnel is filled with acetone and chilled to −50° C. by the addition of dry ice. A piece of E. and D. No. 615 paper, or its equivalent, is placed in the glass funnel.

The filtrate (fraction 2) should be rechilled to −50° C. to ensure complete removal of the high-titer fraction, followed by refiltration through the same paper, if necessary.

The crystals on the paper (fraction 3) are rinsed three or four times with 25-ml. portions of petroleum ether which have been chilled to −50° C., the rinsings being combined with the filtrate. The filter paper containing the high-titer components (fraction 3) is transferred to a funnel at room temperature, and the crystals are dissolved by repeated rinsings with warm petroleum ether.

The filter paper containing fraction 1 is washed with ethyl ether. A small amount of material that belongs in this fraction may not have been removed in the first filtration and will be precipitated instead with fraction 3, but will not then dissolve in petroleum ether; hence, a small residue may remain after fraction 3 is dissolved in warm petroleum ether. This residue should be dissolved in ethyl ether and added to the ether solution of fraction 1.

The solutions of these three fractions may be transferred to tared beakers, the solvent evaporated with carbon dioxide,

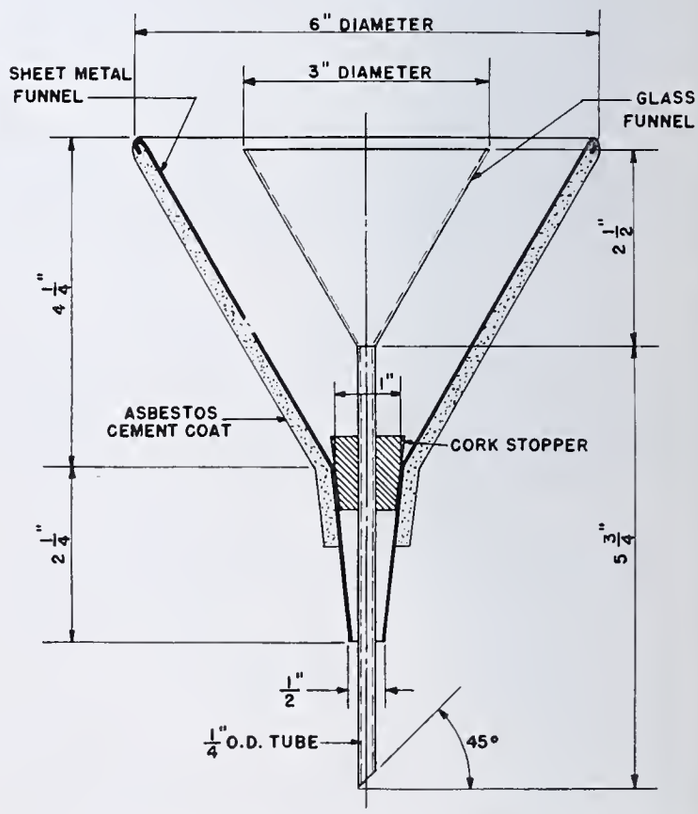

Figure 1. Jacketed Funnel

TABLE II. STEARIC-OLEIC ACIDS

	Stearic Taken	Oleic Taken	High-Titer Found	Low-Titer Found
	%	%	%	%
Mixture 1	72.2	27.8	74.0	26.0
2	48.3	51.7	48.7	51.3
3	32.8	67.2	32.6	67.4

	Mixture 1			Mixture 2			Mixture 3		
Fraction	Original	High	Low	Original	High	Low	Original	High	Low
Neutralization No.	205	207	197	201	205	197	196	205	195
Iodine value (Wijs)	29	8	90	47	6	89	66	9	88
Titer, ° C.	47.2	51.7	...	42.2	52.2	...	34.4	51.1	...

TABLE III. STEARIC-NAPHTHENIC ACIDS

	Stearic Taken	Naphthenic Taken	High-Titer Found	Low-Titer Found
	%	%	%	%
Mixture 4	71.1	28.9	71.8	28.2
5	53.5	46.5	53.0	43.3
6	26.4	73.6	26.3	70.6

	Mixture 4			Mixture 5			Mixture 6		
Fraction	Original	High	Low	Original	High	Low	Original	High	Low
Neutralization No.	203	208	206	208	210	205	206	209	206
Iodine value (Wijs)	11	8	27	11	9	28	15	8	27
Titer, ° C.	43.3	52.8	...	39.4	52.8	...	26.7	52.8	...

and the weights of each found. Iodine value, acid number, etc., may be determined on each fraction. As shown in Table I, the high-titer fraction (No. 3) may contain elaidinized unsaturated acids. If these are absent, the iodine value of fraction 3 should be below 15.

It has never been found necessary to repeat the extraction on the low-titer fraction (No. 2), or to repeat more than once on fraction 3. If after one recrystallization the iodine value of the high-titer acids is still over 15, the presence of elaidinized fats is indicated.

When the high-titer fraction (No. 3) predominates, sharp separation is aided by reducing the temperature first to about −10° C. and filtering the crystals, then lowering the temperature to −30° C. and finally to −50° C. The successive filtrations may use the same paper. The temperature of the funnel should be the same as that of the solution.

Suction has been found to give slower filtration than gravity, possibly because the crystals are wedged into the pores of the paper by the additional force. Suction also draws warm air through the crystals and may melt them.

The percentages of the three fractions in a mixture of fatty acids may be determined to 5 per cent or less with one separation, and to within 0.5 per cent with one recrystallization of the high-titer fraction (No. 3).

The time elapsed between weighing out the mixed fatty acids and weighing each of the three fractions is approximately one hour.

The importance of fraction 1 depends upon the nature of the sample. Thus the presence of castor oil, degras, etc., may be indicated by this fraction. However, other oxidized or hydroxylated acids may be present because of oxidation during storage or overheating. Another source of fraction 1 may be the saponification itself, as of dichlorostearic acid.

Petroleum ether is preferred to the acetone recommended by Brown and Stoner (*1*) because of the insolubility of condensed moisture therein. On a humid day, this condensation is practically impossible to prevent when working at −50° C. With petroleum ether, however, no difficulties are encountered. Ether gives almost as much trouble as acetone.

Experimental

KNOWN MIXTURES. The following examples illustrate the accuracy obtainable by the above method.

Stearic Acid–Oleic Acid. Triple-pressed stearic acid and a high-grade commercial oleic acid (which showed no high-titer components by the above method) were weighed in varying proportions, blended, and segregated, with only one crystallization. The results are given in Table II. A second crystallization of the high-titer fraction, when it is over 40 per cent of the mixture, would have increased the accuracy of the separation.

Stearic Acid–Naphthenic Acid. This illustrates the dependence of the method on titer, not on degree of saturation of the acids. The naphthenic acids were purified by distillation. The results, given in Table III, indicate a loss of naphthenic acid during the removal of the petroleum ether.

Stearic Acid–Rosin. Rock rosin was used in this separation. Since this contained some material insoluble in petroleum ether (fraction 1), the tests on the low-titer fraction given in Table IV do not agree with those of the original rosin.

From these, and similar experiments, Table I has been compiled. Acids below lauric have not been investigated.

ACIDS FROM NATURAL FATS. Table V shows typical results obtained from samples of fats. For the fish oil, the Hanus iodine value is given. The high-titer fraction of this fat was recrystallized, but the iodine value remained at 24. The presence of an elaidinized fatty acid is thus indicated.

The tallow whose tests are shown in Table V can be blended in a spindle oil to the extent of 10 per cent and this blend will remain clear at 15° C. Another tallow was encountered, however, which precipitated from a similar blend at 55° C. The conventional tests on the separated fatty acids gave no explanation, but a segregation showed that a low-grade or slop tallow had been blended with a high-titer tallow, or with tristearin, to give an artificial material with apparently natural tests. The data are given in Table VI.

NATURAL FATS. Conventional methods of fat analysis involve a separation of the fatty acids, with tests of the acids. The tallows shown in Tables V and VI indicate that two fats whose acids appear to be similar in tests may be different in their oil-solubility, to mention one characteristic. A more extreme example is a mixture of olive oil and tristearin whose separated fatty acids show a neutralization number, iodine value, and titer identical with a conventional tallow. Such a mixture is a hard, brittle cake, obviously different from the soft, pasty tallow.

A knowledge of the make-up of a fat may be obtained by a

TABLE IV. STEARIC ACID–ROSIN

Stearic Taken	Rosin Taken	High-Titer Found	Fractions 1 and 2 Found
%	%	%	%
31.2	68.8	31.4	68.6

Fraction	Original	High	Low	Rosin
Neutralization No.	172	208	164	162
Iodine value (Wijs)	108	9	143	132
Titer, ° C.	...	52.7	...	...
Liebermann-Storch	Positive	Negative	Positive	Positive

TABLE V. NATURAL FATS

	Tallow	Palm Oil	Fish Oil
High-titer, %	47.0	55.2	27.0
Low-titer, %	53.0	44.8	73.0

	Tallow			Palm Oil			Fish Oil		
Fraction	Original	High	Low	Original	High	Low	Original	High	Low
Neutralization No.	195	198	193	207	208	194	195	198	187
Iodine value	52	9	88	48	9	90	150	24	202
Titer, ° C.	37.8	51.1	...	43.3	51.3	...	23.9	49.4	...

TABLE VI. ADULTERATED TALLOW

	Original	High-Titer	Low-Titer
%	...	53	47
Neutralization No.	197	198	194
Titer, ° C.	40.5	37.9	...
Iodine value	54	10	112

TABLE VII. TALLOW

	Original	High-Titer	Low-Titer
Fraction, %	..	40	60
Iodine value (Wijs)	54	31	65
	Approximate Composition		
	%		%
Tristearin	18	Free fatty acid	
Distearin, monoolein	32	Oleic	95 plus
Monostearin, diolein	46	Stearic	5 minus
Triolein	4		

segregation of the fat itself. Typical data are given in Table VII for a sample of tallow (not the same as that shown in Table V). While Tables II, III, and IV may be employed to show the fallacy of the use of iodine values to calculate the composition of a mixed fat, this has been done in Table VII to give an approximate composition. It was assumed that tri- and distearin constituted the high-titer fraction, while tri- and diolein formed the low-titer component. A more accurate result could be obtained by segregating the fat as shown, then obtaining the separated fatty acids from each fraction, and segregating them. The term "stearin" and "olein" may include homologs of stearic and oleic acids.

A similar segregation of the "adulterated tallow" (Table VI), with similar calculations, shows a higher percentage of both tristearin and of triolein, with smaller amounts of the intermediate compounds. The mixture of olive oil and tristearin indicates its composition definitely when separated in this manner.

This segregation method may also be applied to a fat as such in order to determine the nature of the free fatty acid. From the acid numbers of the original fat and of the high- and low-titer fractions, it was found that the free acids of the tallow of Table VII were over 95 per cent oleic acid. A less direct method also may be used, titrating the free acids, extracting them as soap, liberating them as acids, and segregating.

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Quantitative Determination of Phosphorus in Phosphor Bronze

A Spectrographic Study

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An investigation of the spectrographic determination of phosphorus in copper-tin alloys which have been deoxidized with Phos-copper is described. The essential requirement of arc currents of high amperage, of the order of 10 amperes, is demonstrated. The effect of other factors on the characteristics of the arc is presented, and photometric methods of measuring line intensities are discussed.

The resulting arc temperatures eliminated the use of solid metallic electrodes and required the use of the standard regraphitized carbon electrodes with a 200-mg. sample.

The proposed method makes possible the spectrographic determination of 0.001 per cent of phosphorus in these alloys, with an accuracy of ±5 per cent.

THE increasing application of the spectrograph to quantitative determinations of compositional variations of industrial raw materials has been very striking in the past decade, although applications appear to have been relatively few with respect to certain insensitive elements, particularly carbon, sulfur, and phosphorus. Phosphorus was selected for study, since for many years it has been the major deoxidizer for the copper-tin alloys. Such alloys carry percentages of phosphorus particularly applicable to spectrographic determination, roughly in the range from a trace to 0.50 per cent.

A review of the literature reveals a lack of information on the spectrographic analysis of phosphorus. One reason for this is probably found in a list of arc sensitivities of 50 elements developed by Ryde and Jenkins (*11*) in which phosphorus was assigned an arc sensitivity between 2 and 3. By definition, therefore, a sensitivity of 2 is equivalent to 1 part in 100 or 1 per cent, while that of 3 is equivalent to 1 part in 1000 or 0.10 per cent. Recently Rollwagen and Ruthardt (*10*), reporting on the spectrographic analysis of a platinum alloy containing 0.005 per cent of phosphorus, announced the detection of the phosphorus lines of λ 2535.65 and λ 2553.28 Å. (All wave lengths used are those given by Harrison, *5*.) This result was secured when solid electrodes were excited by a direct current arc of 12 amperes at 110-volt potential. Condensed spark excitation, on the other hand, in the case of a similar alloy containing as much as 0.10 per cent of phosphorus, produced a positive record of the strong line at λ 2535.65 Å., with the next sensitive line 2553.28 Å. barely visible. The arc data more nearly coincide with American practice, since the detection of phosphorus in bronze has been extended beyond the 0.10 per cent claimed by Ryde and Jenkins (*11*).

Subsequent to the initiation of the experimental work, Pierce, Torres, and Marshall (*9*) reported negative results with respect to phosphorus in a sample of pig iron containing by chemical analysis 0.08 per cent of phosphorus. More recently, Gregg and Irish (*4*) have again noted the insensitivity

of carbon, sulfur, and phosphorus in ferrous products. The negative statements previously quoted, together with the absence of any mention of phosphorus determinations in many recent articles on spectrographic analysis of steel and iron products, would appear to have established the insensitivity of that element by this method of analysis.

Despite the apparent general belief in the insensitivity of phosphorus, spectrographically, it was decided to investigate the possibility of extension of the range of determination of this element in low-phosphorus bronzes of the 95-5 or Grade A type.

Apparatus

The spectrograph available for the investigation was of the large Littrow type as manufactured by the Bausch & Lomb Optical Company. The dispersion in the operation range was of the order of 2.5 Å. per mm. The instrument was equipped with a standard Bausch & Lomb 220-volt direct current arc excitation system. However, in the course of experiment, the standard resistance was discarded in favor of a more flexible and extensive one, giving a range from 1 to 20 amperes.

Eastman No. 33 plates were used throughout. All plates were developed under standard conditions of time and temperature, with developer of Eastman D-11 formula. The plates were calibrated by using a standard Bausch & Lomb sector disk of a step ratio of 1 to 1.5, rotated by a constant-speed, split-phase motor of 1800 revolutions per minute. This figure was established by measurements with a tachometer stroboscope.

Excitation

A range of analyzed phosphor bronze samples was generously supplied by the Scovill Manufacturing Company. These samples differed widely in form, as drillings, millings, and thin sheet, and it was early realized that the method of excitation employed must be prepared to accommodate this dissimilarity in form. The choice appeared to be limited to the carbon arc electrode.

Smith (*14*) reported extensively on this method when applied to analysis of impurities in lead. Brownsdon and van Someren (*2*) described a technique using a copper rod 3 cm. in diameter as a positive sample container. Hitchen (*6*) detailed the use of carbon rods in the quantitative spectrographic determination of impurities in tin. It remained for Milbourn (*8*) to investigate comprehensively and establish the increased sensitivity of the globule arc method with a carbon electrode. He recommended a sample between 0.20 and 0.50 gram, using a positive copper electrode and a negative carbon rod-supported globule. In this way, Milbourn was able to detect small percentages of bismuth, arsenic, lead, antimony, and tin, whereas under similar conditions of excitation no traces of these elements were detectable with metallic solid electrodes.

It was concluded from the above evidence that increased sensitivity of phosphorus might be expected from some modification of the globule arc method of excitation, particularly in view of the close periodic group relationship with several of the previously mentioned elements. Preliminary experimental work was initiated with the object of verifying this assumption.

It was decided to examine the effect on the sensitivity of the lines λ 2534.01 Å. and λ 2554.93 Å. when amperage, weight of sample, and length and portion of arc were varied, employing Acheson regraphitized carbon electrodes of 0.78-cm. (0.31-inch) diameter. The positive electrode cups were drilled to a depth of 3.0 mm. with wall thickness of 0.75 mm., then purified by the solution method of Standen and Kovach (*15*). The upper negative carbon electrodes were pointed in a pencil sharpener and similarly purified.

Current in Arc

A series of exposures of six analyzed samples ranging from 0.25 to 0.008 per cent phosphorus, of 250-mg. mass, were excited for 4 minutes, at 4.5 amperes, with the plain sector disk set to expose the plate for one fourth of that interval. The slit width was maintained constant at 15 microns for all exposures. This plate revealed no evidence of phosphorus below a content of 0.10 per cent. It was concluded that increased sensitivity might be obtained at amperages well above the 4.5 employed. Accordingly, sample 3 (0.067 per cent phosphorus) was exposed under similar conditions with the exception of increased amperage. The results were positive. Later, additional plates were taken of alloys of even lower phosphorus content, with the final plate obtained when a new sample of 0.001 per cent phosphorus alloy was exposed at amperages of 8, 10, and 12. This plate indicated that the phosphorus line λ 2534.01 Å. was barely visible for the latter two exposures.

Observations of experimental conditions indicated the essentials of consistent results to be a function of several variables. The effect of increasing the amperage from 10 to 12 is not so great as might be expected, since the negative effect of the mechanical ejection of small globules from the sample is greatly increased. This is not opposed to the findings of Slavin (*13*), who recently correlated the increase in emission with increase in amperage when operating his fed iron arc. Duffendack and his co-workers (*3*) showed the importance of studying the effect of variations in arc current. It was decided to make all future determinations on arcs employing a current of 10 amperes.

Mass of Sample

This factor was studied using a range of samples weighing 5, 10, 20, 50, 100, 200, and 500 mg. Observations made during the excitation period indicated that less mechanical loss was occasioned with the three higher weights. The spectra showed slight choice between the exposures made with 100 and 200 mg. However, better sampling conditions prompted the choice of 200 mg. The 500-mg. sample would require a larger cup. The authors' conditions were definitely unsatisfactory for small weights, since in some cases all of the sample was ejected on striking the arc or after a relatively short portion of the exposure was made. It is possible that smaller-diameter rods and different-dimensioned cups might overcome this difficulty.

Length and Fraction of Arc Registered

Efforts in this direction were confined to several plates. The first investigated the effect of variation of electrode spacings of 1, 2, and 3 mm. This effect was negligible, and since manipulation of the image of the arc on the slit was less difficult at 3 mm., all subsequent exposures were made with that setting. Continual adjustment of the electrodes is required because of their rapid rate of combustion when such high currents are used. Increasing this spacing appreciably beyond this figure diminishes the current amperage with resultant critical diminution in intensity of emission. Evidence was developed to indicate that a reduction of 1.0 ampere between 10 and 9 amperes was sufficient to eliminate all trace of phosphorus lines in spectra produced from arc excitation of samples containing phosphorus of the order of 0.001 per cent.

It was further noted, under the prevailing conditions of excitation, that a period of 15 seconds was required to melt the material and to establish a steady arc. To determine whether this procedure introduced loss of phosphorus, two plates were taken with a continuous series of exposures covering excitation for 8 minutes. The samples selected for this multiple exposure contained 0.008 and 0.19 per cent of phosphorus, respectively. Examination of these spectra showed that for each concentration the line intensities were the same for the first 3 minutes. In both cases, traces of phosphorus were detected even after 5 minutes of excitation. The absence of traces of phosphorus after that period was attributed to the mechanical ejection of the globule. The persistency of phosphorus lines in the spectra of both series was surprising.

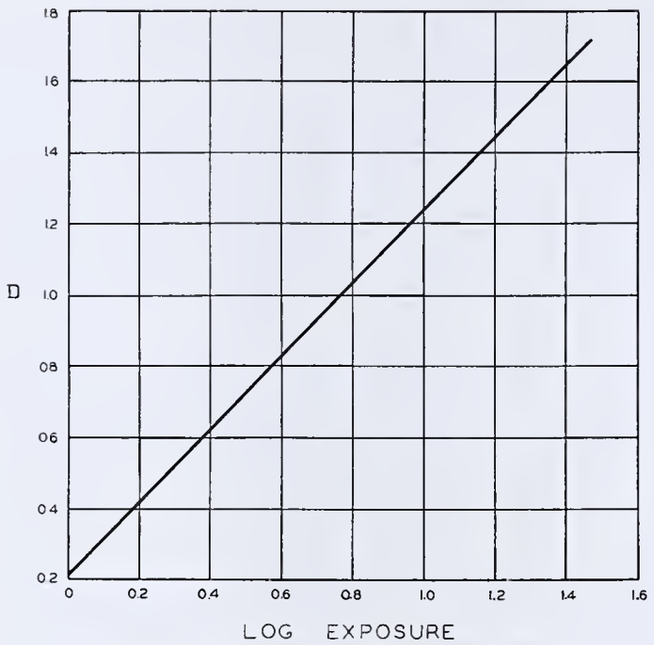

FIGURE 1. TYPICAL BLACKENING CURVE

Summary of Excitation and Development

The foregoing results were interpreted to indicate the following method of operation.

A 200-mg. sample of material, drillings, millings, or otherwise, was weighed out on an analytical balance, and transferred to a 0.78-cm. (0.31-inch) regraphitized rod, which was cupped to a depth of 3 mm. with wide walls of 0.75 mm. This container was made the lower and positive electrode while the negative electrode was a rod, similar in composition and diameter, but pointed. The space between electrodes was carefully adjusted to 3 mm. and the rheostat adjusted to yield an arc of 10 amperes. The arc was struck and the final adjustment of current was made to give exactly 10 amperes on the ammeter scale. The plate exposure was started after an interval of 15 seconds and continued for 1 minute. A constant current of 10 amperes was maintained throughout the exposure period.

The final spectrum of each plate was for calibration and was obtained by exposure of a similar sample but with the step sector rotating in front of the slit.

All plates used were of the standard Eastman 33 type and were developed for 5 minutes with Eastman D-11 developer at a temperature of 18.3° C. (65° F.). (Mechanical loss is always encountered in excitation of this high current density, but it was believed from observation that this effect was reduced to a constant minimum value.)

Microphotometer Plate Calibration

Scribner (*12*) has reviewed the available methods of comparing spectral line intensities. The increased accuracy and rapidity of modern photometric comparison methods appear to be well established, and this method was adopted. All final photometer readings were secured through the kindness of the Chase Copper and Brass Works on their Hilger nonrecording photoelectric microphotometer. The microphotometer readings for Figure 2 are given in Table II.

The adoption of this method required the use of some plate calibration procedure. Thomson and Duffendack (*18*) have described a method using a step slit, while Strock (*17*) has detailed a step sector procedure. The latter was selected, and the step sector employed is described above.

A review of the literature reveals that the conversion of blackness readings of the plate to intensities has been treated in several different ways. Mankowich (*7*) evolved one procedure in which he defined opacity as equal to:

$$\frac{\text{clear plate reading} - \text{zero reading}}{\text{molybdenum line reading} - \text{zero reading}}$$

when determining molybdenum in an alloy steel. A working curve was then plotted using log opacity against log concentration. Brode (*1*) adopted a method of plotting log of intensity ratios against log of concentration. In this case, intensity is clear plate deflection less line image deflection, with the intensity ratio that between the unknown and an internal standard line. Strock (*17*) established what he designated as a blackening curve in which

$$D \text{ (density)} = \log \frac{I_0}{I}$$

where D is blackening or optical density, I_0 is the galvanometer deflection when this reading represents a measure of the difference between a completely unexposed portion of the plate or clear plate and of the zero setting of the instrument or that for a completely exposed portion of the plate (maximum blackness), and I is the difference between zero setting and the reading for the blackening of the image of the desired line. From the microphotometer readings, values of D are calculated and are plotted against the log of the relative exposures. These are obtained by calculation involving the use of the characteristic factor of the step sector and the particular step. Such a curve is shown in Figure 1, while the plotted data appear in Table I.

TABLE I. BLACKENING

Step (No. 1, smallest opening)	1	2	3	4	5	6	7	8
Relative exposure	1.00	1.50	2.25	3.38	5.07	7.60	11.1	16.7
Log of exposure	0	0.176	0.352	0.529	0.705	0.881	1.045	1.223

In order to compensate for slight variations in excitation and photographic conditions, it is customary to plot the log of the intensity ratio of a line of the unknown against a line of some internal standard element. The region of the spectra in which the strong lines of phosphorus are located, is not particularly favorable for the selection of a suitable comparison line. However, several lines of the major elements (copper and tin) were selected and measured photometrically and the log of the resulting intensity ratio was plotted. Only three of the sensitive phosphorus lines, λ 2534.01, λ 2553.28, and λ 2554.93 Å., were used. The line λ 2535.65 Å. was discarded because of the proximity of the high intensity iron line, λ 2535.604 Å.

The most consistent results were obtained when the intensity of the copper line λ 2441.6 Å. was used as a comparison

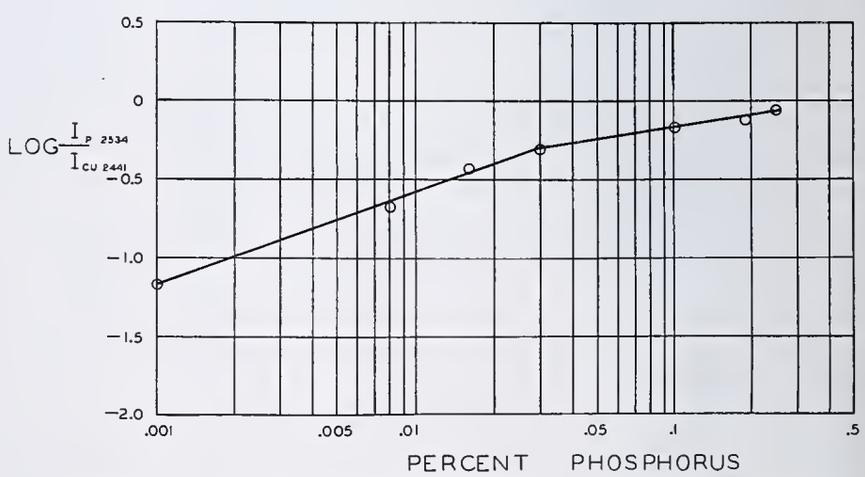

FIGURE 2. WORKING CURVE FOR DETERMINATION OF PHOSPHORUS

standard. This line is not entirely free from objections; it would have been more desirable at somewhat longer wave length, although there is no other closer copper line. The intensity is relatively high as compared to that of the phosphorus lines. On the other hand, the existence of 95 per cent of the alloy as copper ensures freedom from variations due to changes in composition. There are several lines of tin in this region, but these were discarded, since it was planned to investigate subsequently alloys of lower tin content.

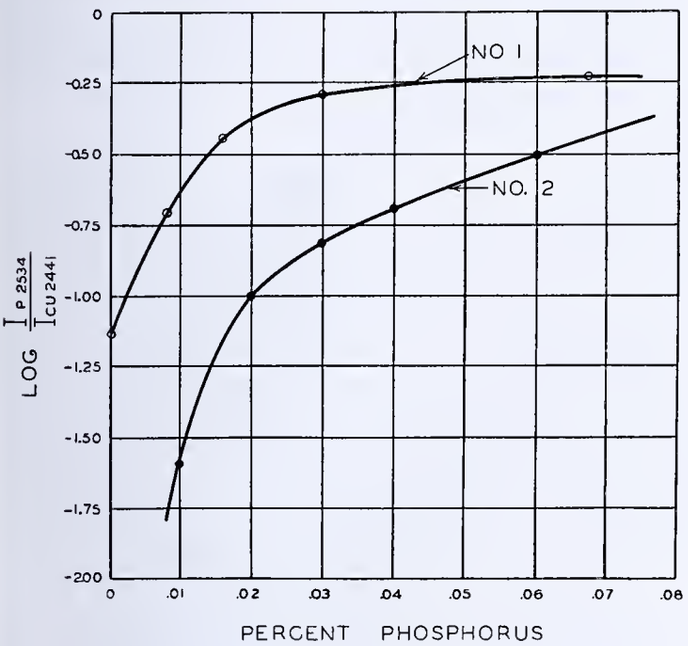

FIGURE 3

After the blackening curve for a plate was constructed, the D values of the measured spectral lines were calculated from the microphotometer readings. Using these calculated values the intensities of the lines were taken from the blackening curve, and then intensity ratios of the desired lines were calculated. The log of these intensity ratios was then plotted against per cent concentration. The working curve was made by plotting $\log \frac{I_P}{I_{Cu}}$ against log of per cent phosphorus. Three of these curves were developed using the three previously mentioned phosphorus lines against the same value for the copper line λ 2441.6 Å.

Discussion of Curve

All three phosphorus lines yielded working curves of satisfactory value. However, since the curve obtained on plotting $\log \frac{I_{P\ 2534}}{I_{Cu\ 2441.6}}$, which is the weakest line of phosphorus, was usable at the authors' lowest concentration, and the resulting plot more nearly approached a straight line, that curve only has been shown in Figure 2. Data for the curve appear in Table II.

TABLE II. DATA FOR WORKING CURVE

(Data for $\frac{I_{P\ 2534}}{I_{Cu\ 2441.6}}$: I_0 = 35.4 cm., log I_0 = 1.549)

	1	2	3	4	5	6	7
P, %	0.001	0.008	0.016	0.030	0.100	0.190	0.250
P, deflection, cm.	17.7	11.5	6.6	4.1	2.3	1.9	1.3
P, log of deflection	1.248	1.061	0.819	0.613	0.362	0.279	0.114
D (calcd.)	0.301	0.488	0.730	0.936	1.187	1.270	1.435
I_P (from Fig. 1)	0.09	0.28	0.50	0.70	0.89	1.03	1.19
Cu, deflection, cm.	0.9	0.9	0.8	0.7	0.8	0.7	0.8
Cu, log of deflection	0.046	0.046	0.097	0.155	0.097	0.155	0.097
D (calcd.)	1.595	1.595	1.646	1.704	1.646	1.704	1.646
I_{Cu} (from Fig. 1)	1.35	1.35	1.39	1.45	1.39	1.45	1.39
I_P/I_{Cu}	0.067	0.207	0.360	0.482	0.640	0.710	0.855
Log I_P/I_{Cu}	1.174	0.684	0.444	0.317	0.194	0.149	0.068

This approach to a straight-line condition is lost through bending of the curve at a concentration of above 0.03 per cent phosphorus. Apparently, the high amperage excitation current so essential to produce any trace of phosphorus in alloys of lower content is no longer necessary.

Strock (*16*) discussed in great detail the factors which contribute to such variations, emphasizing the dependence of exposure on two factors—time and the intensity of the light. This explains the authors' ability to detect traces of phosphorus in the original experimental plate when the content was in the range above 0.03 per cent and the excitation amperage was approximately 4.5.

Figure 3 shows two curves, plotting $\log \frac{I_{P\ 2534}}{I_{Cu\ 2441.6}}$ against concentration. Curve 1 is that for standard phosphor bronze alloy, while No. 2 is a phosphorized Admiralty, containing 28.0 per cent zinc, approximately 1.2 per cent tin, and small quantities of phosphorus. This shifting of the curve has been attributed to the presence of high percentages of zinc. Similar phenomena have been obtained by many workers in this field.

Conclusion

The determination of the quantitative spectrographic analysis of phosphorus in phosphor bronze has been investigated. Evidence indicates the possibility of estimating phosphorus in amounts of the order of 0.001 per cent or higher, using the globule arc method with high excitation amperage. An accuracy of ±5 per cent is claimed for this method.

Acknowledgments

The authors wish to express their gratitude to W. B. Price, F. M. Barry, and R. W. Bailey of the Scovill Manufacturing Company for their gift of analyzed samples; to B. H. McGar and P. A. Leichtle of the Chase Copper and Brass Works for permission to use their microphotometer; and to P. A. Leichtle for his kind service in measuring some of the plates.

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Determination of Surface Areas

Pigments, Carbon Blacks, Cement, and Miscellaneous Finely Divided or Porous Materials

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DURING the last few years a method for measuring surface areas of iron synthetic ammonia catalysts by means of low-temperature adsorption has been developed (*1*, *6*, *7*) and extended in its application to other metallic catalysts and to a variety of nonmetallic adsorbents including soils (*8*), powdered bacteria (*13*), silica gel, chromium oxide gel, potassium chloride, anhydrous and hydrated copper sulfate, Darco G, Darco B, and activated charcoal (*2*). Since the method is comparatively simple and rapid in operation and appeared from previous work to be rather widely applicable, it seemed worth while to attempt to utilize it in measuring the surface area of a number of industrially important finely divided materials. The present paper contains a summary of the results obtained on a series of carbon blacks, zinc oxide, pigments, titanium dioxide, barium sulfate, zirconium silicate, graphite, cement, lithopone, paper, and cuprene.

Although a detailed recapitulation of this method for measuring surface areas need not be included here, a few theoretical and experimental aspects of its nature are briefly summarized. The surface areas are obtained by selecting on each experimental adsorption isotherm of some gas such as nitrogen the point corresponding to a monolayer. A multiplication of the number of molecules required to form the single layer by the average area occupied by each adsorbed molecule yields a numerical value for the absolute area of a given weight of the absorbent.

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The absolute surface area values obtained obviously depend upon the accuracy with which the point corresponding to a monomolecular adsorbed layer can be picked. Originally this point (designated as point B) was selected empirically as the lower pressure extremity of the long linear portion of the experimental adsorption isotherm.

As an illustration, point B on the isotherm for the adsorption of nitrogen on "arrow black" has been indicated in Figure 1. The experimental evidence that led to the selection of B on the iron catalysts was threefold. A series of isotherm determinations for different gases on a single catalyst showed better agreement among the values for the catalyst area if B was considered as the volume of gas in a monolayer than if any of the other likely points on the isotherms were chosen (*6*). Secondly, the heats of adsorption calculated by the Clapeyron equations from a series of isosteres showed that the heat of adsorption at B was intermediate between probable values for first-layer and second-layer heats of adsorption. Thus a selection of a volume 25 per cent greater than that at B yielded a heat of adsorption close to that of liquefaction of the gas, whereas a volume 25 per cent smaller yielded a heat of adsorption at least 50 per cent greater than the heat of liquefaction and clearly characteristic of adsorption in the first layer. Finally, the chemisorption of a layer of carbon monoxide over the entire surface of the pure iron catalyst required a volume of carbon monoxide approximately equal to the volume of physically adsorbed gas at B on the low-temperature isotherms (*6*). These three separate types of evidence convinced the authors that the volume of gas corresponding to B on the isotherms was close to that required for a monolayer.

An excellent confirmation of the selection of B as the monolayer and a valuable new working tool in using this method of surface area measurements are contained in a recent paper (*3*) on the theory of multilayer adsorption such as is apparently being obtained in isotherms of the type shown in Figure 1. It was shown that the isotherms could be plotted in such a manner as to yield a straight line whose slope is closely the reciprocal of the volume of gas required to form a monolayer.

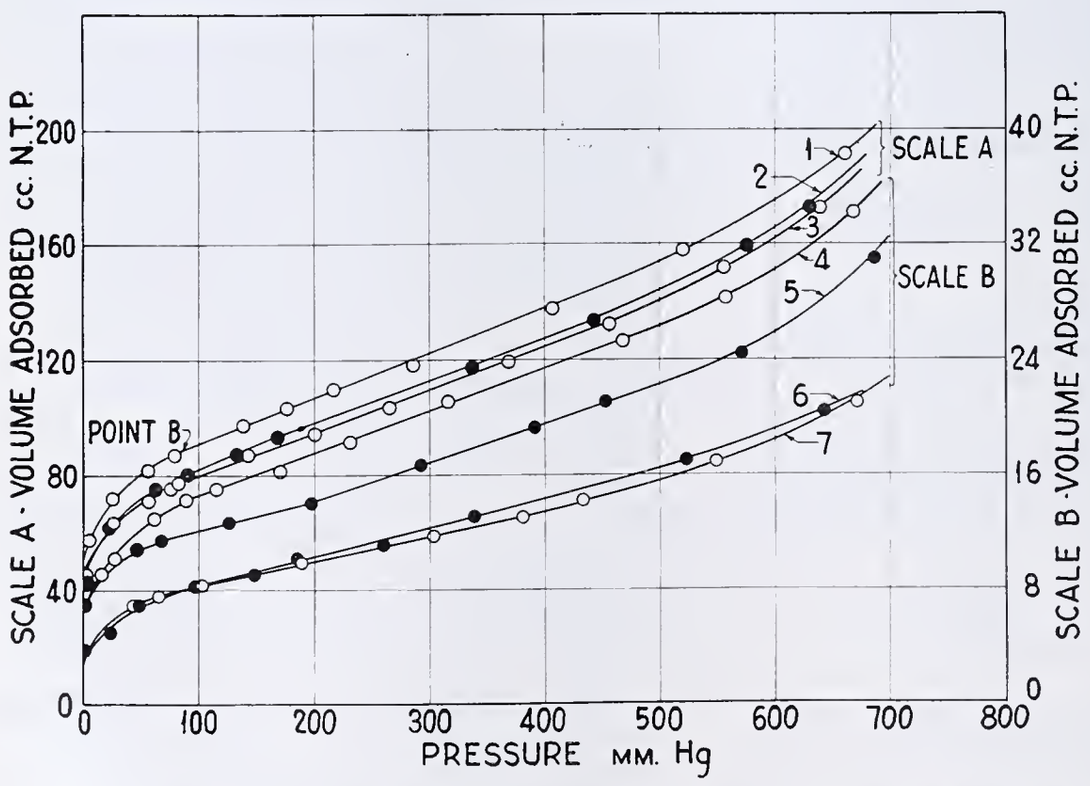

FIGURE 1. ADSORPTION OF NITROGEN ON CARBON BLACKS AT LIQUID NITROGEN TEMPERATURES

1. 3.29 grams of arrow black
2. 3.09 grams of Micronex
3. 2.95 grams of Wyex
4. 2.86 grams of P-33
5. 0.792 gram of acetylene black
6. 5.50 grams of thermatomic
7. 4.44 grams of Thermax

The complete equation is

$$\frac{p}{v(p_0 - p)} = \frac{1}{V_m C} + \frac{C - 1}{V_m C}\frac{(p)}{(p_0)} \quad (1)$$

where v is the volume of gas adsorbed at pressure p at a temperature at which the vapor pressure of the liquefied gas is p_0; V_m is the volume of gas in cc. required to form a monolayer; C is a constant related exponentially to the difference

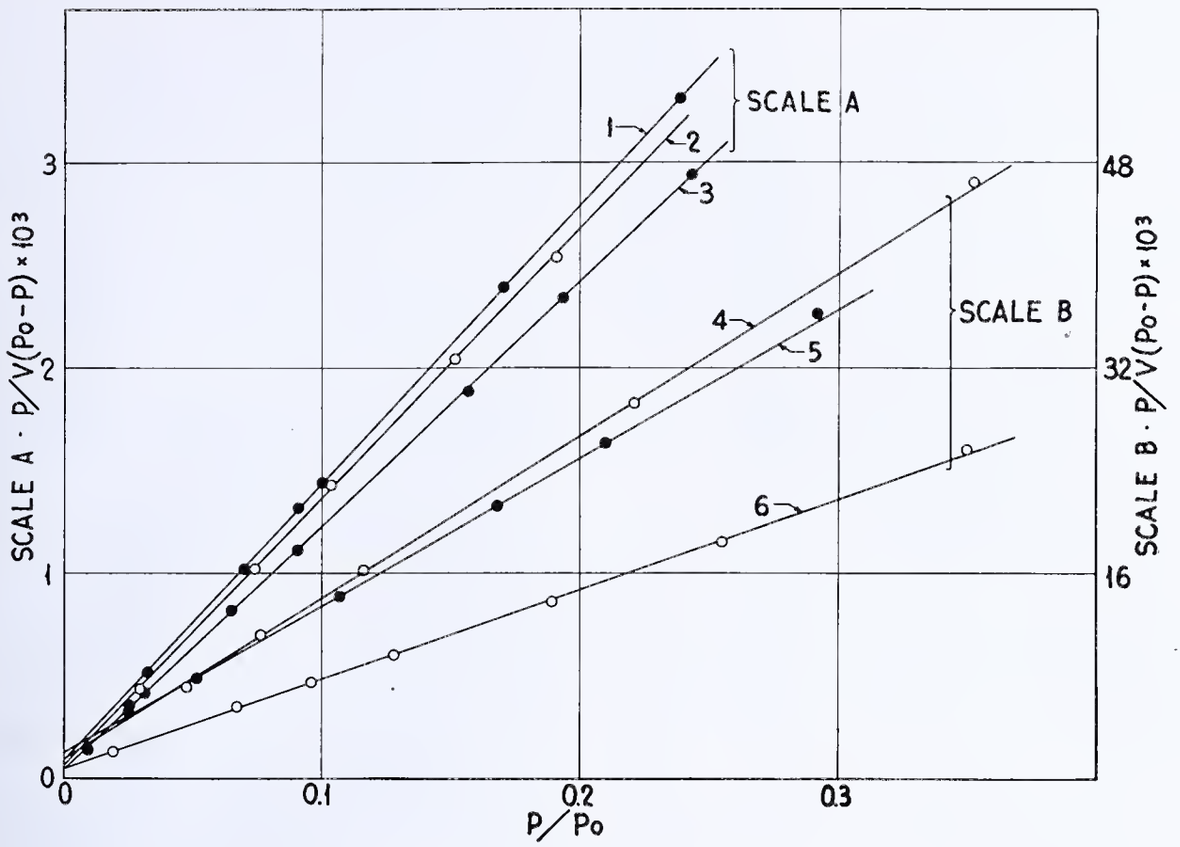

FIGURE 2. ADSORPTION DATA FOR NITROGEN ON CARBON BLACK ACCORDING TO EQUATION 1

1. Wyex
2. Micronex
3. Arrow black
4. Thermax
5. Thermatomic
6. P-33

Weights of samples same as in Figure 1

between the heat of liquefaction of the adsorbate and its heat of adsorption. It is evident that from the slope and intercept of a plot of $p/v(p_0 - p)$ against p/p_0 both V_m and C can be evaluated.

Application of Equation 1 to several hundred isotherms obtained during the last few years shows excellent agreement between the volume of gas adsorbed at B and the volume, V_m. The linearity of the isotherms plotted according to Equation 1 extends up to a relative pressure, p/p_0, of about 0.35. This is sufficient for evaluating V_m. A typical set of such plots is shown for the carbon black samples in Figure 2 and discussed below.

If a material is known to give an S-shaped low-temperature nitrogen adsorption isotherm, only a few adsorption points need to be determined in the relative pressure range 0.05 to 0.3 to fix approximately the slope of plots of Equation 1 and hence to yield values for V_m. In fact, a single adsorption point in the above-mentioned pressure range will, when connected with the origin on such a plot, give a line whose slope will usually differ by less than 5 per cent from that drawn with the help of a number of adsorption points.

The second factor upon which the absolute values obtained for the surface areas will depend is the area occupied by each adsorbed molecule. In a previous publication (*6*) it has been pointed out that two convenient and reasonable molecular area values are those corresponding to the packing of the molecules in the solidified or liquefied adsorbates. Cross-sectional molecular areas calculated from the solidified gases are in general about 20 per cent smaller than those obtained by analogous calculations on the liquefied adsorbates. Absolute values for surface areas are therefore uncertain by at least this amount, though experience has shown that relative areas of materials are reproducible to a few per cent.

Apparatus and Procedure

In the present measurements nitrogen was ordinarily used as adsorbate; in a few experiments, butane was used. The cold baths for these two gases were, respectively, liquid nitrogen and ice. The apparatus and procedure have been described in detail (*1, 6*); a standard adsorption apparatus was used, the adsorption bulb being calibrated with pure helium at the temperature of the cold bath immediately preceding the runs on a given adsorbent.

To remove water vapor and other gases, each sample of material was evacuated for an hour at 100° C. except cuprene and two samples of paper which were pumped out at room temperature. Temperatures of the liquid nitrogen bath varied but little during a run; nevertheless, they were measured periodically by an oxygen vapor pressure thermometer, the vapor pressure data for nitrogen being taken from Dodge and Dunbar (*5*), and those for oxygen from Giauque, Johnston, and Kelley (*11*). The exact temperatures were used in preparing plots according to Equation 1. The "liquid nitrogen" temperatures referred to in the captions of Figures 1 to 6 were always between 77.3° and 79.5° K.; the exact temperature depended, of course, on the oxygen content of the commercial liquid nitrogen. The adsorbate was tank nitrogen dried by passage through a tube of phosphorus pentoxide. As pointed out in a previous paper, a few tenths of a per cent of oxygen in the nitrogen will not interfere with the measurements on any material other than those capable of combining with or chemisorbing oxygen at the temperature of the runs. The butane, a commercial sample from the Ohio Chemical Co., was liquefied, freed from noncondensable gases by evacuation, and partially vaporized. The middle portion of liquid from this vaporization was chosen; its vapor pressure at 0° C. was 777 mm., indicating that the isobutane content must have been small.

Results

CARBON BLACKS. A series of seven carbon blacks used in the present experiments included well-known commercial carbons: Micronex, P-33, arrow black, Wyex, Thermax, thermatomic carbon, and acetylene black. The first six were kindly furnished by the Rubber Division of the National Bureau of Standards and the acetylene black, by Shawinigan Chemicals Limited.

The adsorption isotherms for nitrogen on these seven carbon blacks are shown in Figure 1 and the plots according to Equation 1, in Figure 2. The values for V_m and for the surface areas are summarized in Table I. The areas are calculated by multiplying the value of V_m by 4.38 and dividing by the weight of the sample. The value 4.38 for the area covered by 1 cc. of adsorbed nitrogen is calculated from the density of liquid nitrogen (*6*).

GRAPHITE, TITANIUM DIOXIDE, BARIUM SULFATE, AND ZIRCONIUM SILICATE. In the course of this work Dr. Boyd of the University of Chicago sent the authors samples of graphite, titanium dioxide, barium sulfate, and zirconium silicate, upon which he had made surface area measurements by studying the adsorption of salicylic acid from solution. One of the materials, barium sulfate, had also been measured by the radioactive indicator method by Professor Kolthoff of the University of Minnesota.

The isotherms obtained are shown in Figure 3. The plots by Equation 1 for these and the remainder of the runs are not published because of space limitations; in all cases, however, they were linear throughout the usual range of 0.05 to 0.35 relative pressure and were used to obtain V_m, from which the area in each instance was calculated. The results of the authors' measurements are summarized in Table II, together with the surface area values determined by the other methods mentioned above, and surface area values calculated from plots of the data for butane according to Equation 1. The value of V_m per gram is multiplied by 8.69 to obtain the area in square meters per gram when butane[1] is used as adsorbate.

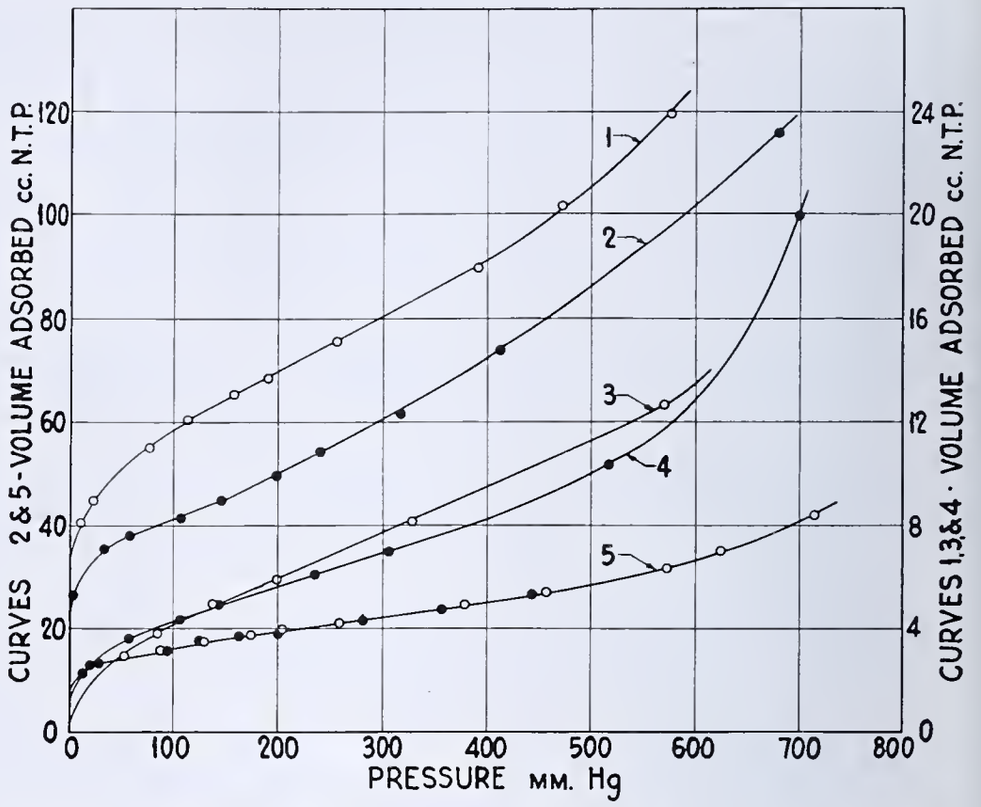

FIGURE 3. ADSORPTION OF NITROGEN AND BUTANE ON FINELY DIVIDED MATERIALS

1. N_2 on 17.52 grams of $ZrSiO_4$
2. N_2 on 5.45 grams of graphite
3. Butane on 6.60 grams of TiO_2
4. Butane on 14.88 grams of $BaSO_4$
5. N_2 on 14.88 grams of $BaSO_4$ (closed circles) and N_2 on 6.60 grams of TiO_2 (openc ircles). Nitrogen runs are at liquid nitrogen temperatures; those for butane, at 0° C.

[1] The authors would like to call attention to an error in Table III in the paper of Brunauer, Emmett, and Teller (*5*). The value for V_m in column 4 for butane should be 44.7 instead of 58.2 cc. per gram. In columns 5 and 6 the surface area values should be 387 for butane and in column 8 the value of $E_1 - E_L$ should be 930 instead of 1930 calories. The error arose from using scale *A* instead of scale *B* in Figure 2 of that paper in calculating the value of V_m and the constant *C* for the butane isotherm.

TABLE I. SURFACE AREA MEASUREMENTS ON CARBON BLACK SAMPLES

Material	Weight	V_m	Area	Diameter of Average Particle	
				By adsorption	By ultramicroscopic count[a]
	Grams	*Cc.*	*Sq. m./g.*	*Micron*	*Microns*
Micronex	3.08	75.2	106.7	0.031	0.061
P-33	2.86	14.42	22.12	0.151	0.159
Arrow black	3.29	84.8	112.7	0.029	...
Wyex	2.95	74.2	110.2	0.030	...
Thermax	4.44	7.8	7.69	0.43	...
Thermatomic carbon[b]	5.497	8.54	6.81	0.49	1.12
Acetylene black	0.792	11.68	64.5	0.052	0.130

[a] Gehman and Morris (*10*).
[b] Names here used were those on samples received by authors. However, thermatomic carbon and Thermax are probably one and the same thing, "thermatomic carbon" being used ordinarily to designate a class of carbons formed by thermal decomposition of hydrocarbon gases.

TABLE II. SURFACE AREA MEASUREMENTS

Material	Weight	V_m by Nitrogen	V_m by Butane	Area Calculated from: Adsorption of nitrogen	Adsorption of butane	Adsorption of salicylic acid	Radioactive indicator method
	Grams	*Cc.*	*Cc.*	*Sq. meters per gram*			
Graphite	5.447	38.22	..	30.73	..	3.96	..
TiO_2	6.602	14.91	5.00	9.88	6.58	5.55	..
$ZrSiO_4$	17.52	11.05	..	2.76	..	1.33	..
$BaSO_4$	14.86	14.60	4.60	4.30	2.68	1.73	2.2

TABLE III. SURFACE AREA MEASUREMENTS ON ZINC OXIDE PIGMENTS

Sample No.	Weight of Sample	V_m	Area	Average Particle Diameter
	Grams	*Cc.*	*Sq. m./g.*	*Microns*
F-1601	5.492	11.89	9.48	0.115
K-1602	6.444	12.95	8.80	0.124
G-1603	7.403	6.56	3.88	0.28
KH-1604	17.167	2.58	0.658	1.65

TABLE IV. SURFACE AREA MEASUREMENTS ON LITHOPONE AND CEMENT

Material	Weight	Point *B*	V_m	Area
	Grams	*Cc.*	*Cc.*	*Sq. m./g.*
Lithopone				
Before calcining and grinding	12.587	100	..	34.8
Calcined but not ground	8.324	2.6	..	1.37
After grinding	6.244	4.9	..	3.43
Standard cement	15.456	3.8	3.87	1.08[a]

[a] This is to be compared with the value 0.1890 sq. meters per gram designated by the Bureau of Standards for use in calibrating Wagner turbidimeter.

ZINC OXIDE. Four samples of standard zinc oxide pigments on which measurements had previously been made by other methods were kindly furnished by the New Jersey Zinc Company. They are designated as samples F-1601, K-1602, G-1603, and KH-1604, and are the same as samples 1, 2, 4, and 5, respectively, of the paper by Ewing (*9*). The isotherms obtained are shown in Figure 4; the results are tabulated in Table III.

CEMENT AND LITHOPONE. One sample of cement furnished by the National Bureau of Standards was measured. It was standard sample from lot 1140 and is used for calibrating the Wagner turbidimeter. The isotherm is shown in Figure 5, together with those for samples

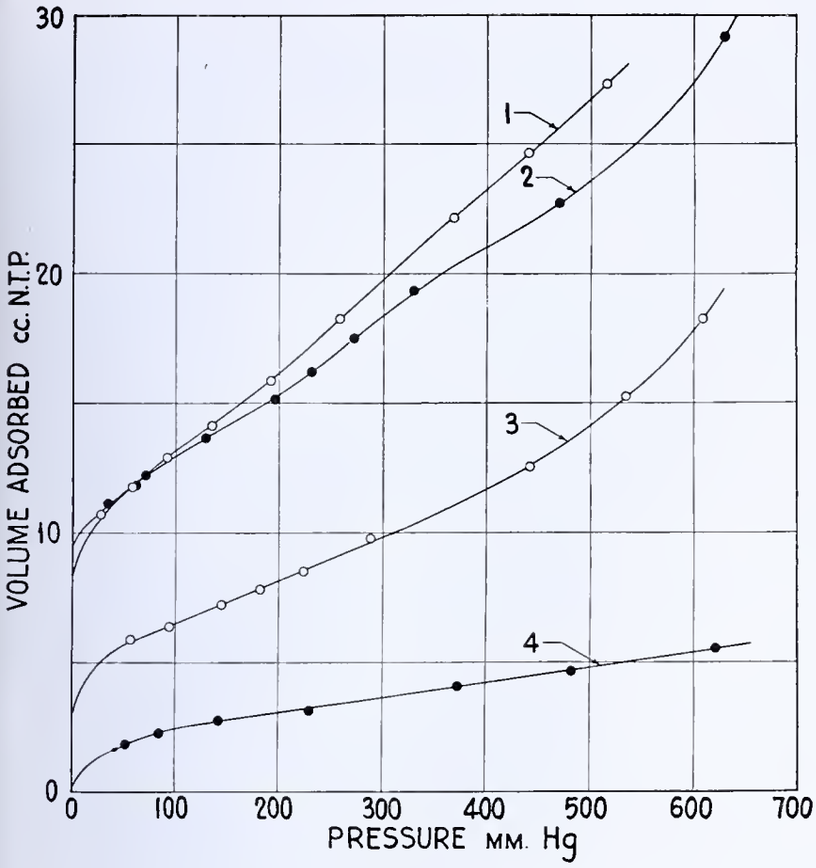

FIGURE 4. ADSORPTION OF NITROGEN ON ZINC OXIDE SAMPLES AT LIQUID NITROGEN TEMPERATURES

1. 6.44 grams of sample K-1602
2. 5.49 grams of sample F-1601
3. 7.40 grams of sample G-1603
4. 17.2 grams of sample KH-1604

of lithopone selected at three different points in a manufacturing process. Curve 1 is for the precalcination stage, curve 4 for the calcined lithopone, and curve 2 for the calcined sample after the final grinding. The area of the cement sample tabulated in Table IV was calculated from a plot of the data according to Equation 1; the areas of the lithopone samples were obtained by selecting B on the adsorption isotherms. Plots by Equation 1 could not be made from the data on the lithopone samples, since, at the time the runs were made, an accident to the oxygen thermometer prevented measurement of the exact value of the temperature and hence of P_0, the vapor pressure of liquid nitrogen.

PAPER AND CUPRENE. The isotherms, together with the surface area values for two samples of paper and one of cuprene, are shown in Figure 6. The two samples of paper are of the type used as insulation in telephone cables; they were measured both after being evacuated at room temperature and at 100° C. The cuprene was furnished by Shawinigan Chemicals Limited; the paper, by J. B. Whitehead of Johns Hopkins University.

Discussion of Results

The particle diameters (in microns) calculated from the surface areas obtained for the various samples of carbon are compared in Table I with those published by Gehman and Morris (*10*) based on ultramicroscopic measurements. A density of 1.80 has been taken for the density of carbon in calculating the particle size from the surface areas. In view of the fact that their carbon samples were prepared prior to 1932 and that those in the present paper were obtained in 1939, the agreement between their results and the authors' seems remarkably good. The ratio of the average particle size obtained by their method to that obtained from the nitrogen isotherms varies from 1.06 to 2.5 on the four samples studied by both methods. In general, one would expect the particle size obtained by the low-temperature adsorption method to be smaller, if anything, than that obtained by the ultramicroscope, since the lower limit of the size that can be detected by the latter method is about 0.01 micron, whereas the adsorption method ought to apply equally well to smaller particles. Recent determinations of the size of Micronex carbon black particles by use of the electron microscope yield values of 28 millimicrons (*14*). This value is in excellent agreement with the value 31 millimicrons obtained in the present work (Table I) for a different sample of Micronex.

In Table V are listed the results of five methods for calculating the diameters of the particles of zinc oxide in the four samples studied. The values for all but the gas adsorption method were furnished by the New Jersey Zinc Company. All their results but the ultramicroscopic have been summarized recently by Ewing (*9*). The direct microscopic examination yields values for both S, the surface area in square meters per gram, and N, the number of particles per gram of material. Both adsorption methods yield values for S, whereas the ultramicroscopic method

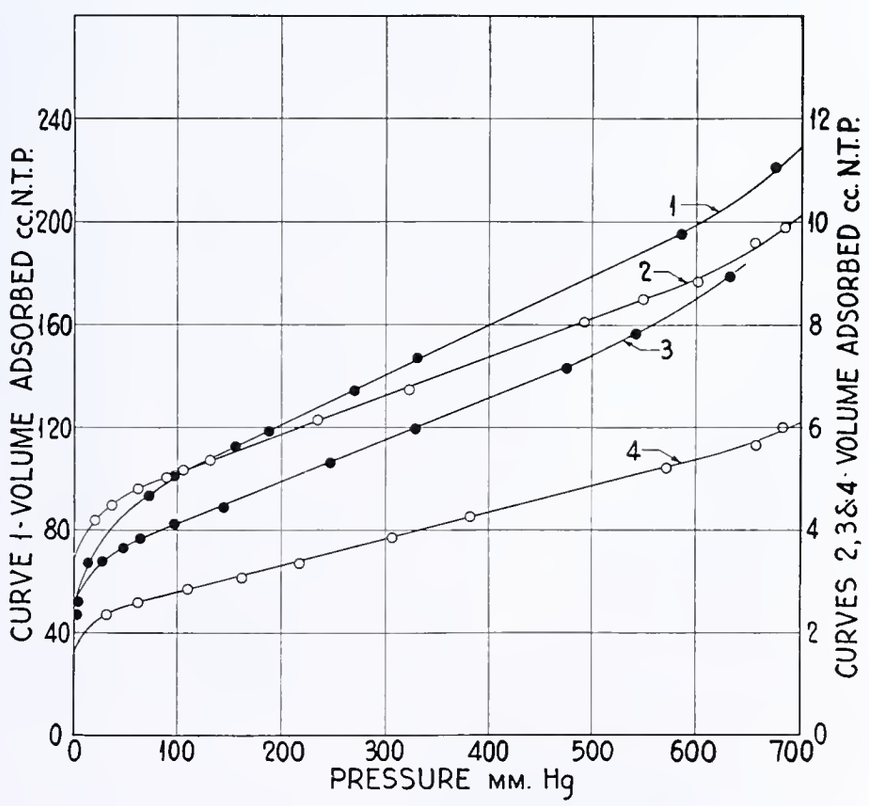

FIGURE 5. ADSORPTION OF NITROGEN ON LITHOPONE AND ON A STANDARD CEMENT SAMPLE AT LIQUID NITROGEN TEMPERATURES

1. 12.59 grams of lithopone before calcination and grinding
2. 6.24 grams of lithopone-finished product
3. 15.46 grams of a standard cement sample
4. 8.32 grams of lithopone after calcination but before grinding

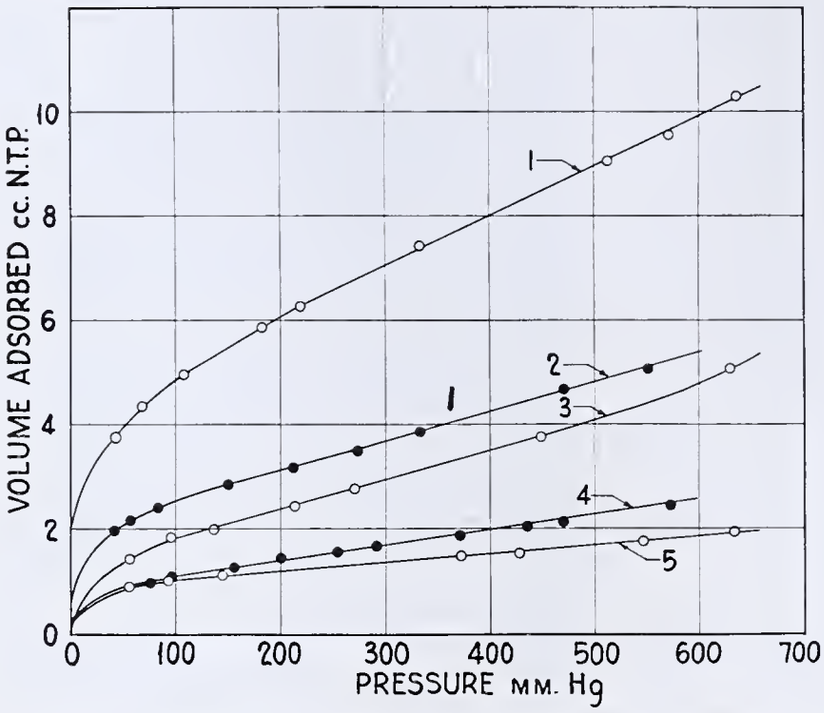

FIGURE 6. ADSORPTION OF NITROGEN ON PAPER AND ON CUPRENE AT LIQUID NITROGEN TEMPERATURES

1. 13.41 grams of paper D evacuated at 25° C.
2. 0.542 gram of cuprene
3. 13.41 grams of paper D evacuated at 110–115° C.
4. 8.02 grams of paper A evacuated at 25° C.
5. 8.02 grams of paper A evacuated at 110–115° C.

Calculated surface areas for samples for curves 1, 2, 3, 4, and 5 are 1.59, 20.7, 0.654, 0.606, and 0.568 sq. meters per gram, respectively.

yields values for N. The average diameters of the particles expressed in microns are calculated from values for S or N in an obvious manner by the equations included in Table V, using for the density of zinc oxide the value 5.60 grams per cc.

Several interesting comparisons can be noted. In the first place, the average diameters as judged by the nitrogen adsorption method are in all cases smaller than those obtained by adsorption from solution by a factor of 2 or 3. This is not unexpected and conforms to the results of measurements on titanium dioxide, barium sulfate, zirconium silicate, and graphite whose surface areas had also been measured by the adsorption of salicylic acid from solution. On all the samples of zinc oxide except KH-1604, the reheated oxide, the average diameters obtained by the nitrogen adsorption method are smaller than those yielded by the direct microscopic counts of either N or S. This, too, would be expected both because the direct microscopic methods probably do not take into account particles smaller than about 0.2 micron and because the "sorption surface", by including the internal surface of any pores that may be present, will tend to be larger than the external surface measured microscopically.

The values for the particle diameters of zinc oxide obtained by the ultramicroscope and by the nitrogen adsorption method are in remarkably close agreement, as can be seen from Table V. The first three oxides listed give especially close agreement, the values for the diameters by the nitrogen method being, on the average, a little smaller than, but less than 20 per cent different from, those obtained with the help of the ultramicroscope. Of course, a close comparison of d_3 and D in Table V cannot be made because of uncertainty as to the numerical value of the shape factors involved (*12*). Only for the reheated zinc oxide, sample KH-1604, is the average particle size appreciably larger by the nitrogen method than by the ultramicroscopic method. For this oxide the value for D, calculated from N by direct microscopic examination is likewise somewhat smaller than that obtained from the nitrogen isotherms. The explanation for this is probably to be found in the technique used in preparing samples for microscopic or ultramicroscopic examination. In the former instance the rubbing down process involved in preparing the material for slides will tend to break up groups of particles that have fused together during the period of reheating the zinc oxide to high temperature. The dispersion technique required for preparing the ultramicroscopic samples is even more likely to break up such aggregates, since the zinc oxide for such samples is actually compounded into rubber and then recovered by dissolving away the rubber. It is reasonable to expect, therefore, that the particle size will be smaller by the ultramicroscopic method for this one sample than by the nitrogen adsorption method. The fact that the direct microscopic value is also smaller than that obtained by the nitrogen adsorption method is understandable as a balance between two factors involved in the former, the breaking up of particles in preparing the slides, and the missing of some of the particles because of the impossibility of observation below 0.2 micron.

Ordinarily the breaking up of agglomerates would not affect the apparent particle size as judged by the low-temperature isotherms, because the individual particles in agglomerates are sufficiently loosely held together to give ready access to the nitrogen molecules used as measuring units. Only when the agglomeration consists of an actual sintering together of a number of particles as by a high-temperature treatment such as used on sample KH-1604 will the area of the agglomerate be considerably less, as judged by the low-temperature isotherms, than the sum of the surface areas of the particles that together make up the agglomerate.

The results shown in Table II are self-explanatory. The reason for the larger discrepancy between the values for graphite obtained by adsorption from solution and from the

TABLE V. SURFACE AREA MEASUREMENTS ON ZINC OXIDE

Pigment	No.	Direct Microscopic d_3	Direct Microscopic S	Adsorption of Methyl Stearate d_3	Adsorption of Methyl Stearate S	Adsorption of Nitrogen d_3	Adsorption of Nitrogen S	Number of Particle Measurements: Direct Microscopic D	Direct Microscopic $N \times 10^{-10}$	Ultramicroscope D	Ultramicroscope $N \times 10^{-10}$
Kadox Black Label-15	F-1601	0.28	3.87	0.19	5.54	0.115	9.48	0.21	2040	0.135	7260
XX Red-72	K-1602	0.34	3.27	0.24	4.56	0.124	8.80	0.25	1140	0.16	4200
XX Red-78	G-1603	0.79	1.37	0.55	1.97	0.28	3.88	0.49	153	0.26	1075
Reheated zinc oxide	KH-1604	1.86	0.58	4.50	0.24	1.68	0.66	1.40	6.6	0.82	32.0

S Surface area, square meters per gram.

d_3 Average diameter (microns) related to S by $S = \frac{6}{\rho d_3}$.

N Number of particles per gram $\times 10^{-10}$.

D Average diameter related to N by $N = \frac{1}{\rho D^3}$. In present table D is expressed in microns.

ρ Density of ZnO particles in grams per cc. = 5.60.

gas phase than for the other three materials is not clear. It presumably points to a large "internal" surface area in cracks or crevices that are inaccessible to large organic molecules in solution and yet are covered by nitrogen molecules during the low-temperature gaseous adsorption measurements. The areas calculated from the butane isotherms are usually considerably smaller than those obtained from nitrogen isotherms. This is consistent with previous experience with this gas (*2*, *3*, *6*); the explanation of the smaller values is not yet certain. Small symmetrical molecules appear in general to be preferable to large long molecules for surface area measurements.

The area of the standard sample of cement had been determined by the usual liquid flotation method and was given to the authors as 0.1890 sq. meters per gram. This is smaller by a factor of about 6 than the value 1.08 sq. meters per gram obtained in the present adsorption studies. The authors prefer not to discuss the possible causes of this discrepancy until more work has been done comparing the two methods. However, in view of the general agreement of the nitrogen adsorption method with ultramicroscopic methods for carbon black and zinc oxide, the error is probably to be looked for in some of the assumptions made in the standard determinations by elutriation methods.

Close examination of the adsorption isotherms on the zinc oxide samples and on acetylene black will reveal some shape peculiarities analogous to those noted previously for certain iron synthetic ammonia catalysts (*2*). The linear portion of the isotherm whose lower extremity is about 100 mm. in all the present work extends a few hundred millimeters only and then either increases or decreases in slope abruptly before joining the higher pressure part of the curve that is convex to the pressure axis near the saturation pressure. It is not possible to state as yet the cause of these shape irregularities above 400 mm. However, on all of these materials the V_m values obtained from plots of Equation 1 are in good agreement with the point B values.

The runs on lithopone, paper, and cuprene merely serve to illustrate the possible utility of the new method for measuring surface areas of miscellaneous industrial materials. Although due caution should be observed in interpreting the results of similar surface area measurements on materials on which the method has not yet been tried, the authors see no reason to doubt the wide applicability of the low-temperature adsorption method in determining the relative and even the absolute surface areas of a variety of materials [for a critical discussion of the method see (*4*)]. So far, out of the several hundred different materials studied only charcoal (*2*) and dehydrated chabazite give other than the S-shaped adsorption isotherm. For reasons that are not entirely clear but appear to be concerned with pore diameters, the one sample of charcoal and the numerous samples of chabazite that have been tried yield Langmuir-type adsorption curves that do not become convex to the pressure axis as pressure approaches the liquefaction value but approach asymptotically a limiting adsorption value.

Acknowledgment

The authors wish to extend their thanks to B. L. Harris for his assistance in making the experimental adsorption measurements on the zinc oxide samples.

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Colorimetric Method for Determination of Nitrite

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A method employing sulfanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride for the determination of nitrite is proposed. These reagents have been found superior to sulfanilic acid and α-naphthylamine, formerly employed, in that the color developed is clearer, reaches its maximum intensity more rapidly, and remains stable for a longer time. A standardized solution of sulfanilamide is substituted for sodium nitrite as a primary standard to obviate the difficulties arising from the instability of the latter.

PROCEDURES for the colorimetric determination of nitrite in foods, water, and sewage have been based on the diazotization of sulfanilic acid by the available nitrite and the subsequent coupling with an agent such as α-naphthylamine (*1*, *2*, *5*). As employed, these methods are open to two objections: (1) The coupling of diazotized sulfanilic acid with α-naphthylamine is relatively slow, requiring from 10 to 30 minutes for full color development (*2*). With α-naphthylamine acetate the color must be read within 30 minutes (*1*). (2) Primary nitrite standards are unstable and difficult to prepare.

It has been found possible to circumvent these difficulties in part by replacing α-naphthylamine with *N*-(1-naphthyl)-ethylenediamine dihydrochloride, the coupling component suggested by Bratton and Marshall (*3*) for sulfanilamide determinations. It has the advantage of being water soluble, decreases the time required for color development to 2 minutes, gives a final color that remains constant for several hours, and is less sensitive to variations in pH, reacting equally well in acid concentrations ranging from 0.1 to 1 *N*.

In place of sulfanilic acid, sulfanilamide (*p*-aminobenzenesulfonamide) has been used. Sulfanilamide of a high degree

TABLE I. RECOVERY OF NITRITE

(Color developed in a final volume of 50 ml.)

Nitrite Content by Assay	Nitrite Content by Sulfanilamide Standard	Recovery
Mg.	*Mg.*	%
0.0515	0.0516	100.2
0.0424	0.0423	99.8
0.0258	0.0258	100.0
0.0241	0.0242	100.4
0.0241	0.0240	99.6
0.0212	0.0210	99.1
0.0129	0.0130	100.8
0.0071	0.0070	98.6
0.0052	0.0053	101.9
0.0028	0.0028	100.0
0.0026	0.0025	96.2
0.0014	0.0013	92.9
0.0013	0.0013	100.0
0.0006	0.0005	83.3

of purity can be obtained; it is more stable than sulfanilic acid both in the dry state and in solution, and it reacts more rapidly in the coupling process.

Finally, inasmuch as the methods for the colorimetric determination of nitrite and of sulfanilamide (*3*, *4*) employ the same fundamental reactions of diazotization and coupling and the concentrations analyzed lie in a comparable range, the substitution of a standardized stock solution of sulfanilamide for the primary nitrite standard was considered practical. Experiments have shown that sulfanilamide and nitrite react stoichiometrically in the presence of a suitable excess of either.

The following method is proposed for the determination of nitrite.

Reagents

SULFANILAMIDE, 0.2 per cent solution in water. This will keep for at least one month in a refrigerator. Winthrop's "Prontylin, sulfanilamide powder repurified for injection" has been found satisfactory.

HYDROCHLORIC ACID, 1 to 1 dilution of the concentrated acid.

SODIUM NITRITE, 0.1 per cent solution (approximate). This solution will keep for one week or longer in a refrigerator.

AMMONIUM SULFAMATE, 0.5 per cent solution in water, obtained from LaMotte Chemical Products Company, Baltimore, Md., Marshall and Litchfield (*4*) have shown that excess nitrite remaining after diazotization of sulfanilamide interferes markedly with the coupling process. To ameliorate this condition they recommend the use of ammonium sulfamate to remove residual nitrite before the addition of the coupling agent.

N-(1-NAPHTHYL)-ETHYLENEDIAMINE DIHYDROCHLORIDE, 0.1 per cent solution in water, kept in dark bottle. Obtained from LaMotte Chemical Products Company, Baltimore. This is referred to below as the "coupling agent".

Standardization of Sulfanilamide Solution

Dry analytical reagent grade sodium nitrite for 24 hours in a desiccator. Dilute a weighed sample of about 1 gram to 100 ml. in a volumetric flask and assay by titration with potassium permanganate according to the U. S. P. XI method (*6*).

A. Employing the assayed sodium nitrite, prepare an accurately known solution containing about 0.005 mg. of nitrite per ml. With a transfer pipet measure 5 ml. of this into each of two 50-ml. volumetric flasks, add 1 ml. of 50 per cent hydrochloric acid and 5 ml. of the 0.2 per cent sulfanilamide solution, and after 3 minutes add 1 ml. of the ammonium sulfamate reagent. (The sulfamate plays no part here but is added to ensure uniformity of treatment.) Two minutes later add 1 ml. of the coupling agent and dilute to volume with water.

B. At the same time into two other 50-ml. volumetric flasks measure 5 ml. of an accurately prepared 1 to 100 dilution of the 0.2 per cent sulfanilamide solution. Add 1 ml. of 50 per cent hydrochloric acid, 1 ml. of 0.1 per cent sodium nitrite, and about 5 ml. of water. Allow to stand 3 minutes, add 1 ml. of ammonium sulfamate solution to destroy excess nitrite, let stand 3 minutes, add 1 ml. of the coupling agent, and dilute to volume.

Samples A and B prepared as above are read against each other in a colorimeter. With a Duboscq-type colorimeter the nitrite equivalent value of the sulfanilamide solution is calculated from the equation:

$$\frac{\text{Reading of A}}{\text{Reading of B}} \times \text{mg. of } NO_2^- \text{ in A} \times 20 = \text{mg. of } NO_2^- \text{ represented by 1 ml. of 0.2\% sulfanilamide solution}$$

Procedure

The solution taken for analysis should be either neutral or acid. Variations in acid concentration between 0.1 and 1 *N* at the time of coupling do not influence the final color. The unknown should contain no more than 0.05 mg. of nitrite and should be limited in volume to no more than 35 ml. To the unknown sample add 1 ml. of 50 per cent hydrochloric acid, 5 ml. of 0.2 per cent sulfanilamide solution, and let stand for 3 minutes. Add 1 ml. of ammonium sulfamate solution. After 2 minutes add 1 ml. of coupling reagent and dilute to volume. At the same time prepare a nitrite standard from the sulfanilamide solution according to the procedure outlined in B. The unknown is read against the standard and the nitrite present in the sample taken is calculated by the equation:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \frac{NO_2^- \text{ value of 1 ml. 0.2\% sulfanilamide}}{20} = \text{mg. of } NO_2^- \text{ in sample}$$

Precision

Known samples of sodium nitrite in water were analyzed according to the method described. Beer's law was found to hold throughout the useful range of color intensity. The proposed procedure gave results as shown in Table I.

It was found that samples containing less than 0.0025 mg. of nitrite in a final volume of 50 ml. gave colors too faint to be read in a photoelectric colorimeter with a reasonable degree of accuracy. If the final volume can be decreased, the sensitivity of the method will be correspondingly greater. Samples containing more than 0.05 mg. of nitrite in 50 ml. should be diluted before analysis, as this represents a color near the upper limits of desirable intensity.

Time Required for Color Development

Color development of aqueous solutions of sodium nitrite subjected to analysis by the method proposed was followed on a Klett-Summerson photoelectric colorimeter at 5-minute intervals for 1.5 hours. The color attained its maximum intensity within 2 minutes and remained constant for 90 minutes. By the end of 3 hours slight fading had occurred.

TABLE II. EFFECT OF VARYING QUANTITIES OF SULFANILAMIDE ON RECOVERY OF NITRITE

(0.0241 mg. of nitrite taken. Color developed in a final volume of 50 ml.)

Sulfanilamide Added	Nitrite Found	Recovery
Mg.	*Mg.*	%
0.10	0.0173	71.8
0.20	0.0231	95.8
0.30	0.0233	96.7
0.50	0.0241	100.0
1.00	0.0240	99.6
2.00	0.0240	99.6
5.00	0.0242	100.4
10.00	0.0242	100.4
50.00	0.0244	101.2

Stability of Sulfanilamide Solution

The nitrite value for a 0.2 per cent solution of sulfanilamide has been found to remain constant for at least one month. To investigate further the stability of sulfanilamide in water a 0.2 per cent solution was divided into two portions, one of which was analyzed immediately while the other was subjected to ultraviolet irradiation (2537 Å.) to the point of marked discoloration. This was considered an artificial aging well beyond the point at which the solution would normally be discarded. A loss of 1.9 per cent was detected in the discolored portion. Solutions which have been kept at room temperature for more than a year have failed to show

any trace of discoloration, but accurate analytical figures over such a period are not available.

Amount of Excess Sulfanilamide Required

The method proposed depends upon the addition of a sufficient excess of sulfanilamide to assure complete utilization of all the nitrite present. In order to ascertain the permissible upper and lower limits of sulfanilamide concentration,v arying quantities were used for the analysis of a constant amount of nitrite. The results obtained are given in Table II.

Approximately 20 mg. of sulfanilamide per mg. of nitrite were required for complete recovery and 2000 mg. per mg. of nitrite do not interfere. The amount chosen in the method is thus satisfactory for all concentrations of nitrite within the practical range.

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Bromination of Phenols by Means of Bromide-Bromate Solution

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IN CONNECTION with a study of the reactivity of phenols toward paraformaldehyde, it was desirable to have a general method for evaluating the potential reaction capacity towards substitution agents of a phenol or mixture of phenols. A bromination procedure was considered the most likely approach to the problem, since it is generally supposed that aqueous bromine will replace hydrogen atoms ortho and para to a phenolic hydroxyl group rapidly and quantitatively, but will leave unaffected hydrogen atoms not so placed.

Bromination has been employed as an analytical tool previously in connection with the problem of the constitution of the phenol-formaldehyde resins by Koebner (*12*), who thus determined the molecular weights of acid-catalyzed phenolic resins (Novolaks), apparently assuming that bromine entered quantitatively into free ortho and para positions in the resin molecules, without affecting them at any other points.

The method under consideration is essentially that of Koppeschaar (*13*). The application of this method to phenol itself was comprehensively examined by Redman, Weith, and Brock (*14*). Phenol-cresol mixtures and Lysol solutions were studied by Järvinen (*11*), who obtained results consistent to within 0.8 per cent of the theoretical value, assuming a more or less arbitrary value for "cresol". Day and Taggart (*9*) found the method to be applicable to several chloro- and nitrophenols, to certain derivatives of salicylic acid, and to resorcinol, β-naphthol, *m*-cresol, and thymol. They found that it was not applicable to *o*-cresol, which could, nevertheless, be determined accurately by direct titration with potassium bromate solution. Beukema-Goudsmit (*8*) also found that *m*-cresol could be determined accurately by the Koppeschaar method but that *o*- and *p*-cresol could not be so determined.

The application of this method to various alkylated phenols, and to certain substituted phenols of particular interest in connection with the problem mentioned above, has apparently not been undertaken previously. Since comparative behaviors were of greatest interest in this connection, a standard analytical procedure was adopted. It soon became evident that the structure of the phenol determined the extent to which it would react with aqueous bromine under these conditions. The generalizations which were thus disclosed will become evident on examination of the data presented below.

Method

Approximately 2 grams of the phenol were weighed into a 125-cc. flask, about 50 cc. of water were added, and the phenol was brought into solution by means of 10 to 15 cc. of 10 per cent aqueous sodium hydroxide. The solution was transferred to a 500-cc. quantitative flask and made up to volume with water. A 10-cc. aliquot was transferred to a 250-cc. ground-glass stoppered iodination flask. To this were added 25 cc. of 0.1 *N* potassium bromate (from a pipet), 10 cc. of 2 *N* potassium bromide, 50 cc. of water, and 2 to 3 cc. of concentrated hydrochloric acid, in the order given. The stopper was inserted quickly and the cup was filled with water. The mixture was allowed to stand for 10 minutes with occasional shaking. Ten cubic centimeters of 10 per cent potassium iodide solution were then added (in such a way as to prevent loss of gaseous bromine while the stopper was removed), and the mixture was allowed to stand 10 minutes longer, with occasional shaking. The sides of the flask were washed down with water, and the iodine liberated was titrated with 0.1 *N* sodium thiosulfate, using starch indicator at the end of the titration. A blank was run on 25 cc. of potassium bromate solution.

The "potential reactivity", *r*, of the phenol is calculated as follows:

$$r = \frac{0.025\,NVM}{W}$$

N = normality of $Na_2S_2O_3$
V = "net" volume of $Na_2S_2O_3$ used
M = molecular weight of the phenol
W = weight of sample

Table I. Reaction with Bromine of Phenols Which Behave Normally

Phenol	Reactive Positions per Mole Calcd.	Found	%
Phenol, U. S. P., redistilled, water white	3.00	2.98	99.4
m-Cresol[a]	3.00	2.98	99.4
3,5-Xylenol[a]	3.00	2.96	98.0
p-tert-Butylphenol[b]	2.00	2.01	100.5
p-tert-Amylphenol[b]	2.00	1.98	99.4
Thymol[c]	2.00	2.02	101.0
2,2-(*p,p'*-Dihydroxydiphenyl)-propane[d]	4.00	4.09	102.3
m-Ethylphenol[a]	3.00	2.95	98.4
p-Bromophenol[b]	2.00	2.00	100.0
2,4-Dichlorophenol[b]	1.00	1.018	101.8

[a] From Eastman Kodak Co.
[b] Furnished by Dow Chemical Co.
[c] Commercial sample purified by crystallization from benzene and heptane, then from pure heptane; m. p. 50–51°.
[d] Commercial sample purified by crystallization from benzene and dioxane, followed by crystallization from pure benzene; m. p. 157–158°.

Results

Phenols Which Behave Normally. These data are summarized in Table I. Included are all phenols which absorb (within 2 per cent) that quantity of bromine which should be taken up on the assumption that only free positions ortho or para to the phenolic hydroxyl are reactive.

Phenols Which Behave Abnormally. The phenols which behave abnormally, with one exception only, give high

results if the assumption be made that substitution is confined to free hydrogen ortho and para to the phenolic hydroxyl. These data are given in Table II.

SALIGENIN. Two samples of saligenin (obtained from Hynson, Westcott and Dunning, Inc.) were examined. The first, an old sample, indicated 2.84 reactive positions per mole, a value which was not changed by several crystallizations from ethylene chloride. The crystallized material, however, retained a salmon-pink color. The second, a new and perfectly colorless sample, gave consistently the value of 2.97 to 2.98 reactive positions per mole. The "calculated" value for saligenin is 2.00.

DIHYDROXYDIPHENYLMETHANE. Dihydroxydiphenylmethane was prepared from phenol and paraformaldehyde by a modification of the method of Harden and Reid (*10*).

One mole of paraformaldehyde was condensed with two moles of phenol in 200 cc. of 95 per cent ethyl alcohol in the presence of 1.5 cc. of concentrated hydrochloric acid. After 3.5 hours at room temperature, 0.5 hour on the steam bath, and 15 hours more at room temperature, water was added and the alcohol and unchanged phenol were steam-distilled from the reaction product. The non-steam-distillable residue was extracted repeatedly with 400- to 700-cc. portions of hot water, and each extract was filtered and cooled separately. All uniformly crystalline products were filtered, dried, and examined separately. Molecular weights were determined cryoscopically in nitrobenzene. Table III summarizes the results.

Both 4,4′-dihydroxydiphenylmethane and 2,4′-dihydroxydiphenylmethane have been isolated from acid phenol-formaldehyde condensations. The former, which is the less soluble in water, has been reported as melting variously from 148–158° C. The latter is reported to melt at 117–118° and is at least twice as soluble in water at room temperature.

TABLE II. REACTION WITH BROMINE OF PHENOLS WHICH BEHAVE ABNORMALLY

Phenol	Reactive Positions per Mole Calcd.	Found	%
o-Cresol[a,b,c]	2.00	2.20 2.21 2.19	110.0
p-Cresol[d,e]	2.00	2.27 2.27	113.5
p-Ethylphenol[f]	2.00	2.28 2.25	113.2
2,3,5-Trimethylphenol[g]	2.00	2.12	106.0
Carvacrol[h]	2.00	2.22	111.0
p-Benzylphenol[i]	2.00	2.53 2.47	125.0
2,5-Dimethylphenol[a]	2.00	2.28	114
3,4-Dimethylphenol[a]	2.00	2.21	110.5
2,4-Dimethylphenol[a]	1.00	1.80	180
2,6-Dimethylphenol[g]	1.00	2.42 2.50	246
3,5,3′,5′-Tetramethyl-4,4′-dihydroxydiphenylmethane[j]	0	2.92	...
4,4′-Dihydroxydiphenylmethane-3,3′-dicarboxylic acid (methylene-disalicylic acid)[k]	2.00	2.62	131
p-Phenylphenol[l]	2.00	2.09 2.10	105
2,4,6-Tri-*tert*-butylphenol[m]	0	0.14	...
p,p′-Dihydroxybenzophenone[n]	4.00	3.25 3.28	82

[a] From Eastman Kodak Co.
[b] 190° b. p. fraction.
[c] 190–190.5° b. p. fraction.
[d] Furnished by the Barrett Co.
[e] Crystallized twice from benzene and petroleum ether; m. p. 35°.
[f] From Dow Chemical Co., crystallized twice from pure heptane, m. p. 42°.
[g] Furnished by Shell Development Co.
[h] Furnished by Givaudan-Delawanna, Inc.
[i] From Eastman Kodak Co., crystallized from benzene and petroleum ether and from benzene and pure heptane; m. p. 83°.
[j] Sample prepared by condensation of 2,6-dimethylphenol with paraform, crystallized twice from aqueous methanol; m. p. 172–173°.
[k] Furnished by Heyden Chemical Co., crystallized twice from aqueous ethanol; m. p. 234–237°.
[l] From Dow Chemical Co., crystallized twice from aqueous ethanol; m. p. 164–165°.
[m] From Gulf Refining Co., crystallized from benzene and from ethylene chloride; m. p. 129–130°.
[n] From Eastman Kodak Co., crystallized twice from aqueous ethanol; m. p. 105–106°.

Discussion

From an examination of Table I, it is obvious that the phenols which behave normally fall into three classes: those that have no alkyl groups substituted in the ortho or para positions (including ordinary phenol), those that have tertiary or secondary (but not primary) alkyl groups substituted in the ortho or para positions, and those that have halogen substituted in the ortho or para (and presumably also the meta) positions.

From Table II, rather definite generalizations can be drawn as to the abnormalities encountered. Apparently, the presence of one primary alkyl group in the ortho or para position to the hydroxyl group of a mononuclear phenol results in the absorption of between 0.1 and 0.3 mole of bromine beyond that required by the simple theory of ortho-para substitution. In the cases of the two diphenylmethane derivatives studied (*p*-benzylphenol and methylene disalicylic acid), between 0.5 and 0.6 mole of bromine was used up beyond that required. (In the case of methylene disalicylic acid, the abnormality may be explained partially by displacement of —COOH by —Br, as is known to take place with salicylic acid and *p*-hydroxybenzoic acid.) For phenols having two primary alkyl groups in the ortho or para positions, the excess absorption of bromine was between 0.4 and 0.7 mole per alkyl group so placed. This behavior was observed in the cases of two mononuclear phenols and a dinuclear phenol which theoretically contains no reactive positions.

Three special cases were encountered. *p*-Phenylphenol absorbed 0.1 mole of bromine in excess of the theory. *p,p*′-Dihydroxybenzophenone absorbed 0.75 mole less than that required. There is no obvious explanation for either of these cases. Moreover, *p,p*′-dihydroxybenzophenone failed to absorb appreciably more bromine, either when allowed to stand in contact with the reagent for considerably longer time at room temperature, or when heated. Thus, after 20 and 30 minutes' contact at room temperature the results indicated 3.40 and 3.39 reactive positions per mole; and after 40 minutes at room temperature followed by 5 and 10 minutes on the steam bath, 3.40 and 3.42 reactive positions per mole were indicated. The third of the special cases is that of 2,4,6-tri-*tert*-butylphenol, which showed a slight capacity for absorption of bromine, but which may not have been entirely free from isomeric or congeneric impurities.

Barring the few exceptional cases encountered, these data lead to a consistent explanation of the high results obtained with many phenols. The most plausible explanation lies in the assumption that bromine is absorbed, under the conditions described above, by a primary alkyl group situated in the ortho or para position to a phenolic hydroxyl. Several observations may be cited against the alternative theory that the excess substitution occurs in free meta positions which are at the same time oriented ortho or para with respect to an alkyl group: (1) There is no reason to suppose that secondary and tertiary alkyl groups should fail to exert a similar degree of directive influence; (2) those phenols which have one meta position blocked should react to a lesser extent than those in which both positions are open, which is not the case; (3) 2,3,5,-trimethylphenol, which has no free meta position, should behave normally, but does not do so. The more plausible explanation would then appear to be that bromination occurs to some extent in the side chain.

There is, of course, a great mass of literature on bromination of phenols, but unfortunately very little work in addition to that mentioned above was done under conditions similar to those used in the present study, where it was desired to find a suitable quantitative procedure.

However, from the early work of Auwers and Anselmino (*3*) it is known that liquid bromine at somewhat elevated

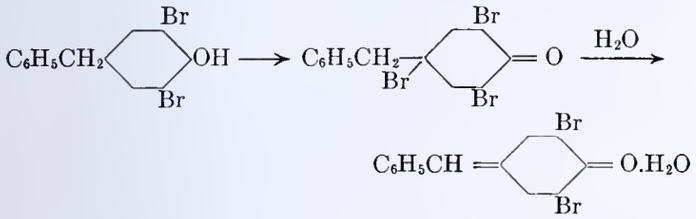
Table III. Dihydroxydiphenylmethane Fractions

Extraction No.	Wt. of Crystals	Melting Point	Mol. Wt. Found	Reactive Positions per Mole Found	Reactive Positions per Mole Calcd.
	Grams	° C.			
1	0.4	152	220	4.62	4.19
2	0.7	151–152	220	4.45	4.19
3	0.4	138–139	220	4.35	4.19
6	1.1	144–147	213	4.13	4.12
8	0.4	147–148	211	4.23	4.10
10	1.1	110–120	223	4.34	4.22
11	0.9	147–148	208	4.03	4.07
		Av.	217	4.30	4.15

temperatures may cause substitution in a methyl group ortho or para to a hydroxyl group, and may cause complete replacement of this group (by bromine) when it is situated in the para, but not ortho, position.

Substitution of bromine in ortho- or para-substituted alkyl groups under various conditions has been reported by other investigators. Typical is the behavior of the cresols, which have been shown by Auwers and co-workers (*2*, *4*, *7*) and by Zincke and Wiederhold (*16*) to give bromomethyl derivatives (pseudobromides) when treated with excess bromine.

The case of *p*-benzylphenol is of special interest in the present work. Zincke and Walter (*15*) found that the "normal" bromination product, 2,6-dibromo-4-benzylphenol, when treated with bromine in carbon tetrachloride and shaken with water, was converted to a methylene quinone, presumably through the intermediation of a "pseudobromide".



The formation of the methylene quinone requires that one mole of bromine react, in excess of that which would normally be anticipated. In the present work, an attempt was made to isolate the methylene quinone or its rearrangement products from a *p*-benzylphenol bromination mixture, but without success, since an uncrystallizable oil was obtained instead.

The cases of saligenin and dihydroxydiphenylmethane deserve special consideration. It is clear that with fresh samples of saligenin, almost exactly 3 moles of bromine are used per mole of saligenin. The simplest explanation of this phenomenon would be that formaldehyde is split off quantitatively by the acid added prior to bromination, and that the product formed is actually tribromophenol. This hypothesis was tested by making up an alkaline solution of saligenin, allowing it to stand for 2 hours, acidifying a portion, allowing this to stand for 10 minutes, and determining the amount of free formaldehyde present. There was found 0.021 mole of formaldehyde per mole of saligenin before acidification and 0.048 mole after acidification. Therefore, if quantitative loss of formaldehyde occurs, it does so during bromination.

Auwers and Büttner (*5*), Auwers and Daecke (*6*), and Autenrieth and Beutel (*1*) studied the bromination of saligenin under various conditions. The products obtained included monobromosaligenin, dibromosaligenin, tribromophenol, and tribromophenol bromide. The last named need not be considered here, since it behaves as a hypobromite, and is therefore quantitatively reduced by reagents such as potassium iodide or sodium thiosulfate. *p*-Hydroxybenzyl alcohol behaved similarly in all respects to saligenin.

By analogy, it seems likely that a methylol group ortho or para to a phenolic hydroxyl group will be removed quantitatively during bromination under the conditions used in this work, or, to put the case a little differently, it will act as though it were not there.

In the case of dihydroxydiphenylmethane, it was suspected that the samples obtained by the procedure described above might contain small amounts of one or more of the congeneric trinuclear phenols. The molecular weights are, indeed, in all cases higher than that calculated for the dimer (200.1), even allowing for reasonable experimental error. The last two columns in Table III are calculated on the assumption that the products are mixtures of the dimer and the trimer in the proportions indicated by the experimental molecular weight. The discrepancy between the average calculated and experimental values is of the same general order of magnitude as that found for simple monoalkylated phenols under the same conditions. The discrepancy vis-à-vis that of *p*-benzylphenol (0.15 mole, as compared to 0.5 mole) is surprising, however. On the whole, it seems clear that an estimation of the molecular weight of dihydroxydiphenylmethane and its polymer-homologs from bromine values will give results at least 3 to 4 per cent low. This, in itself, would possibly not be serious. However, in the case of polymer-homologs derived from alkylated phenols (such as *o*- or *p*-cresol, various xylenols, etc.) the discrepancy between theory and actuality would undoubtedly be much greater, since additional absorption of bromine due to the primary alkyl substituents will certainly occur.

Bromination at Lower Temperature

It seems reasonable to suppose that the competing reactions of substitution of hydrogen by bromine in the ring and in the side chain may have very different temperature coefficients. It might therefore be possible to inhibit the latter effectively without materially altering the course of the former. A number of experiments were therefore carried out at a lower temperature in order to discover under what conditions certain of the para-alkylated phenols took up the theoretical amount of bromine.

Samples of about 2.0 grams each of the para-alkylated phenols were dissolved in 10 cc. of 10 per cent sodium hydroxide and made up to 500 cc., and 10-cc. aliquots were taken for each determina-

Table IV. Titration of Para-Alkylated Phenols at Low Temperature

(t_1 = time of contact with bromine solution. t_2 = time of contact with KI solution)

t_1	t_2	r, Found	r, Calcd.	r, Found (Room Temperature
Min.	*Min.*			
		p-Ethylphenol		
2	5	1.960		
3	3	1.990		
3	5	2.018	2.000	2.25–2.28
4	5	2.047		
6	5	2.075		
8	5	2.083		
		3,4-Dimethylphenol		
2	5	1.933		
3	3	2.030		
3	5	2.040	2.000	2.21
4	5	2.053		
6	5	2.095		
8	5	2.098		
		p-Benzylphenol		
1	1	1.950		
1.5	1.5	1.960		
2	2	2.040		
3	3	2.133	2.00	2.47–2.53
5	5	2.268		
7	5	2.304		
		2,6-Dimethylphenol		
0.25	0.25	1.471		
1	1	2.322		
0.5	5	2.382	1.000	2.42–2.50
2	5	2.445		

tion. Potassium bromate, potassium bromide, and water were added in the usual proportions. The mixture was then cooled to 5° C., 2.5 cc. of concentrated hydrochloric acid were added, and the flask was immediately set in a refrigerator kept at 5° C. After a stated number of minutes, 10 cc. of 10 per cent potassium iodide were added rapidly, and the flask was let stand in the refrigerator for a further period of time. The solution was then titrated rapidly with 0.1 *N* thiosulfate, taking as the titer the volume required to cause a disappearance of the iodine-starch color without recurrence of color on standing for at least 5 minutes. The results obtained are shown in Table IV.

Apparently, then, *p*-ethylphenol and 3,4-dimethylphenol can be estimated with reasonable accuracy and fair reproducibility at 5° C. if the time of contact with the acid bromination solution is around 2 to 3 minutes. *p*-Benzylphenol requires a time of contact under these conditions of less than 2 minutes. The alkyl groups in 2,6-dimethylphenol, on the other hand, are so reactive toward bromine that considerably more than the theoretical amount of bromine is used up even at 5° C. in as little as 15 seconds.

Conditions could probably be established under which many reactive substituted phenols could be titrated reasonably accurately by the Koppeschaar method. However, such conditions would undoubtedly vary from phenol to phenol, and would have to be worked out carefully in each case.

Conclusions

Phenol itself and phenols which contain substituents in the meta position react with acid bromide-bromate solution, causing quantitative substitution at the ortho and para positions. Certain phenols which contain secondary or tertiary alkyl groups in the ortho or para position also brominate quantitatively in this manner. Under the same conditions, phenols which contain primary alkyl groups in the ortho or para positions give results which may be 10 to 150 per cent high, depending on the nature of the substituents. Dihydroxydiphenylmethane also requires somewhat more than the theoretical quantity of bromine solution.

Fresh samples of saligenin require almost exactly three moles of bromine. The methylol group behaves as though it were not present.

p,p'-Dihydroxybenzophenone alone, of the phenols examined, reacted with less than (82 per cent of) the calculated quantity of bromination solution.

Certain phenols having primary alkyl substituents in the para position can be estimated accurately by bromination at low temperature. The conditions required for quantitative bromination must be worked out for each individual alkylated phenol.

Interpretations of the structure and molecular weight of phenolic resins, or related substances, based upon bromination procedure, should be critically reexamined.

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Determining the Maturity of Frozen Peas

A Rapid Objective Method

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CONSIDERABLE work has been published (*2*, *3*, *4*) concerning the determination of the maturity of vegetables by objective methods, mainly for the purpose of establishing methods that are free from the personal element, ever present in the organoleptic method. A large part of this effort was concerned with canned rather than frozen products.

This paper presents an objective method for the grading of frozen peas, to be used in place of the one recommended by the Agricultural Marketing Service. It is an improvement over that method in that it is less time-consuming, less tedious to run in the laboratory, and appears to be more accurate. Like other methods that measure maturity, it does not take into consideration color and appearance defects such as spots and splits.

It depends upon the determination of the specific gravity of thawed peas by means of the difference between the weight of the sample in air and the weight in a liquid, in this case a mixture of xylene and carbon tetrachloride, the specific gravity of which is 1.000. Xylene alone can be used, if desired, although the calculations are a little less simple. This is a modification of the method of Nichols and Reed (*5*) devised for use on prunes, which uses a mixture of xylene and carbon tetrachloride of specific gravity 0.900.

$$\text{Specific gravity} = \frac{\text{weight in air} \times \text{specific gravity of xylene mixture}}{\text{difference of weights in xylene mixture and in air}}$$

The equipment employed by Nichols and Reed was modified for convenient use with the relatively smaller samples of fresh vegetables—about 110 grams or a little larger. A triple-beam balance weighing to 0.1 gram is very good; it can be supported on a stand or shelf and the basket can be attached to the hook under the pan. The sample basket was made of 16-mesh brass screen 8.125 cm. (3.25 inches) high and 5.625 cm. (2.25 inches) in diameter.

The samples of peas are thawed and then drained for 2 minutes before starting the work. The specific gravity can be found as follows: The peas are weighed in air. Their weight in the xylene mixture can be determined by subtracting the weight of the basket in the xylene mixture from that of the peas and basket in this same liquid. The weight in air minus the weight in the xylene mixture gives the difference of weight in this mixture.

The data in Table I were determined at the normal laboratory temperature of 20° C. It is important, however, that the specific gravity of the liquid used remain constant, and it can be adjusted to this constant value for the prevailing temperature conditions. The data are arranged in the order of specific gravity as determined with corresponding figures for alcohol-insoluble solids, Agricultural Marketing Service maturity rating, and organoleptic rating. The alcohol-

TABLE I. DATA ON FROZEN PEAS

Specific Gravity	Organoleptic Rating for Maturity	A. M. S. Test	Alcohol-Insoluble Solids, %	Specific Gravity	Organoleptic Rating for Maturity	A. M. S. Test	Alcohol-Insoluble Solids, %	Specific Gravity	Organoleptic Rating for Maturity	A. M. S. Test	Alcohol-Insoluble Solids, %
1.056	A−	45	9.74	1.075	A−	46	10.19	1.091[a]	B	45	11.91
1.059	A	46	8.40	1.075	A−	45	10.78	1.091	B−	44	11.72
1.059	A−	46	9.94	1.076	A	45	9.56	1.091[a]	C	38	15.85
1.059	B+	46	9.52	1.076	B	46	9.91	1.092[a]	C	35	16.76
1.059	B	45	10.14	1.077	A	42	10.09	1.092[a]	B−	42	14.00
1.060	A	43	9.55	1.077	B+	46	11.17	1.092[a]	C	40	15.36
1.061	A	47	10.42	1.077	B	45	10.46	1.094[a]	B−	42	13.58
1.061	A−	45	10.83	1.078	B	46	10.43	1.096[a]	B−	36	14.68
1.061	A−	44	10.14	1.078	B	46	10.96	1.097	B−	42	12.53
1.062	A−	47	9.62	1.080	B	39	12.53	1.098[a]	C+	41	13.45
1.063	A−	45	9.48	1.080	B−	39	12.13	1.098[a]	C	36	15.04
1.064	A	46	9.81	1.080	B	42	11.09	1.099[a]	B−	41	13.44
1.065	A−	42	11.02	1.080	B	42	11.24	1.099[a]	C+	36	15.73
1.065	A−	44	10.00	1.081	A−, B+	46	10.95	1.099[a]	C	36	15.91
1.066	B+	40	12.08	1.082	B+	40	11.74	1.099[a]	C	36	16.43
1.068	B	44	11.05	1.085[a]	C+	39	14.66	1.100[a]	B−	41	14.03
1.071	B+	40	10.67	1.085	B	34	12.52	1.100[a]	C+	37	15.30
1.072	A−	43	10.60	1.086	A−	46	9.74	1.100[a]	C	36	16.82
1.073	A	45	10.14	1.086[a]	B−	39	15.12	1.103[a]	C	35	14.43
1.073	B+	42	9.94	1.086[a]	C	35	16.65	1.103[a]	C	35	16.85
1.074	A	47	9.96	1.088[a]	B−	36	11.56	1.106[a]	C	34	17.07
1.074	B	40	12.74	1.089[a]	B−	41	13.40	1.106[a]	C+	20	18.12
1.075	A−	44	10.73	1.089[a]	C	34	16.20	1.120[a]	C	33	16.90

[a] Samples removed by brine separator. Some were standards, others substandard rejects.

TABLE II. COMPARATIVE RATINGS

A. M. S. Test	Organoleptic Rating for Maturity	A. M. S. Test	Organoleptic Rating for Maturity	A. M. S. Test	Organoleptic Rating for Maturity
47	A	44	A−	40[a]	C
47	A	44	A−	39	B
47	A−	44	A−	39	B−
46	A	44	B	39[a]	B−
46	A	44	B−	39[a]	C+
46	A−	43	A	38[a]	C
46	A−	43	A−	37[a]	C+
46	A−	42	A	36[a]	B−
46	A−, B+	42	A−	36[a]	B−
46	B+	42	B+	36[a]	C+
46	B+	42	B	36[a]	C
46	B	42	B	36[a]	C
46	B	42	B−	36[a]	C
46	B	42[a]	B−	36[a]	C
45	A	42[a]	B−	35[a]	C
45	A	41[a]	B−	35[a]	C
45	A−	41[a]	B−	35[a]	C
45	A−	41[a]	B−	35[a]	C
45	A−	41[a]	C+	34	B
45	A−	40	B+	34[a]	C
45	B	40	B+	34[a]	C
45	B	40	B+	33[a]	C
45[a]	B	40	B	20[a]	C+

[a] Samples removed by brine separator. Some were standards, others substandard rejects.

insoluble solids were run after first processing the thawed peas in sealed tin cans at 115° C. (240° F.) for 30 minutes. The cans containing the peas were filled with hot water before sealing. After cooling and draining, the determinations were run on these samples in the usual manner (*3*).

All the samples given the rating of C and C+ and some of those given the B− rating were made up of peas that were rejected by the quality separator for freezing as fancy peas, and many for freezing under any condition.

The organoleptic ratings for maturity can be arranged according to the results of the test of the Agricultural Marketing Service (Table II).

In several samples, the organoleptic rating is at variance with both the specific gravity and the alcohol-insoluble solids, but in most instances the correlation is good.

The organoleptic ratings for maturity after cooking (*1*), column 2 of Table I, together with the values assigned to them for use in the calculation of the coefficients of correlation are: A = first grade, 46. A− = low quality in grade A, 43. A−, B+ = borderline between A and B grade, 42. B+ = high quality in grade B, 41. B = satisfactory, but not first grade, 38. B− = low quality in grade B, 35. C+ = high quality in C grade, 34. C = not satisfactory, 33.

Calculation of the coefficient of correlation shows a value of -0.8168 ± 0.0270 for specific gravity compared with organoleptic tests. The test of the Agricultural Marketing Service, run on the same samples and compared with these same organoleptic tests, showed a value of -0.7029 ± 0.0411. When the alcohol-insoluble solids were compared with the organoleptic tests, the value for the coefficient of correlation was -0.8301 ± 0.0252. According to these results the method known as alcohol-insoluble solids and the specific gravity method described in this paper are about equally good, but the latter method can be run in a considerably shorter period of time. In making these calculations there was little need to separate the figures as to varieties because Thomas Laxton peas showed for all practical purposes the same values as the peas of the Alderman variety, both of which were used in this study. It is probable that sweet peas of different varieties grown in other parts of the country for commercial freezing would give similar results.

The suggested tentative standards for frozen peas, based upon the comparison of the specific gravity values with the organoleptic tests and upon a knowledge of the samples themselves, might be set as follows: Fancy, specific gravity 1.084 and lower; standard, specific gravity 1.085 to 1.094. Samples having a specific gravity of 1.095 and higher should be considered substandard.

These standards can be revised if and when an extra-standard grade is generally packed.

Acknowledgment

The author wishes to thank L. S. Fenn of the Agricultural Marketing Service, United States Department of Agriculture, and his associates for running the organoleptic tests and also for grading these samples on the basis of the test of the Agricultural Marketing Service. In addition, he wishes to thank the General Foods Central Laboratories, Hoboken, N. J., and the Snider Packing Corporation, Rochester, N. Y., for financial assistance, for supplying material used, and for making available several of their plants for this work.

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APPROVED by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 383, June 3, 1940.

CORRECTION. In the article on "Determination of Methionine in Certain Mixtures" by J. J. Kolb and Gerrit Toennies [IND. ENG. CHEM., Anal. Ed., 12, 723 (1940)] the last paragraph on page 724 refers to Table I and should not have been printed as a part of the summary.

A Study of the Moisture in Soybeans

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THE study of moisture in biological materials by oven methods has been attended by several difficulties, among which were the probability of error due to loss or gain in weight during the process of cooling the sample, the considerable work involved in obtaining a series of determinations, and the unknown effect, on a series of determinations, caused by cooling the oven during the removal of samples. Through the development in the U. S. Regional Soybean Industrial Products Laboratory of the apparatus for the continual observation of changes in weight at oven temperatures (*1*), these difficulties and others were eliminated so that a systematic study of the moisture relations of soybeans could be undertaken.

It seemed desirable for such a study to compare several types of oven determinations, several varieties of soybeans, a number of samples of the same variety having a range of protein content, a number of samples having a range of oil content, and a number of samples having different moisture contents.

In the portion of the study reported here, four oven methods—namely, the air oven at 105° and 130° C., and the vacuum oven at 80° and 105° C. under less than 5-mm. pressure—have been investigated with the apparatus (*1*), using five varieties of soybeans. The ovens and temperatures are those commonly used, and the varieties of beans were selected because they differ greatly. The hay-type Peking variety is a small black bean which is recognized as having a very tough seed coat, the vegetable-type Giant Green variety is a very large bean; and the other three varieties, Illini, Manchu, and Dunfield, represent the usual grain type of bean.

In addition to the comparison of the oven methods as applied to these varieties, a comparison was made of the effect of the seed coats by studying the loss in moisture of the whole undamaged seed and that of the whole unground seed with the hull removed.

Treatment of Samples

The samples were conditioned by keeping them for more than 3 weeks in a constant temperature–constant humidity room maintained at 25° C. and at a relative humidity of 50 per cent. Except for the whole-bean study, the samples were ground in a Wiley mill with a 1-mm. screen before being conditioned in the humidity room. The sample for each determination was taken from the humidity room in a weighing bottle to prevent an undeterminable loss during the initial weighing of the sample.

Experimental Results

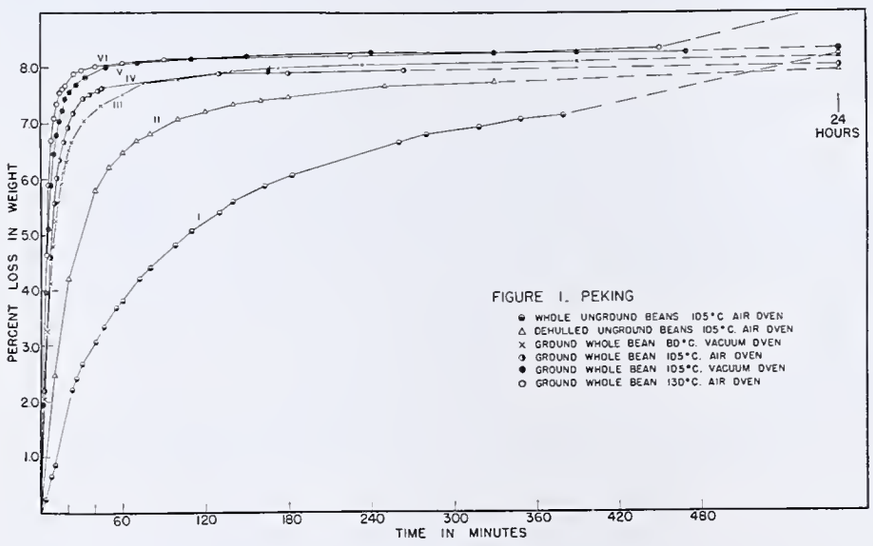
The results obtained for each variety of bean are shown in Figures 1 to 5, with the percentage loss in weight in terms of the entire sample plotted against the time. On this basis, the results of the various methods of analysis on a single variety of bean may be compared.

For a comparison of the results obtained by a single method on several varieties of beans, it is necessary to consider the oil content, since the oil is the only major constituent of the bean which is essentially water-free. The presence of this water-free oil will obscure the actual moisture content of the moisture-containing components and cause a displacement of the entire loss in weight curve. Figures 6, 7, and 8 and Table I were prepared from results calculated to the oil-free basis.

Discussion

Before exploring the inferences of the results, it should be recalled that the several methods of analysis subject the samples to somewhat different conditions. The surrounding atmosphere in the vacuum oven methods has a very low humidity, while in the air oven and circulating air oven the surrounding atmosphere has an appreciable moisture content due to external atmospheric conditions. The same relative situation exists in the two types of ovens with regard to the oxygen content of the atmospheres.

It might be expected that the difference in humidity in the atmosphere in the ovens would be enough to cause significant differences in the equilibrium moisture content arrived at by



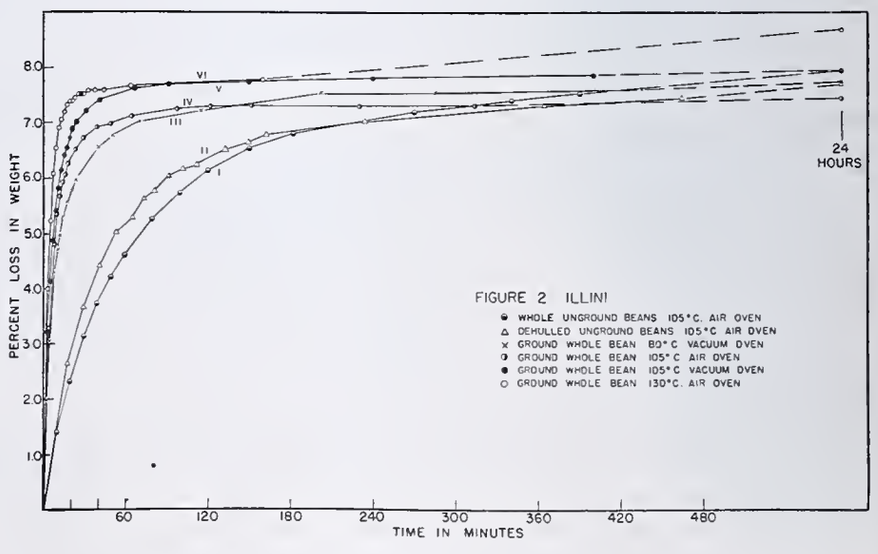
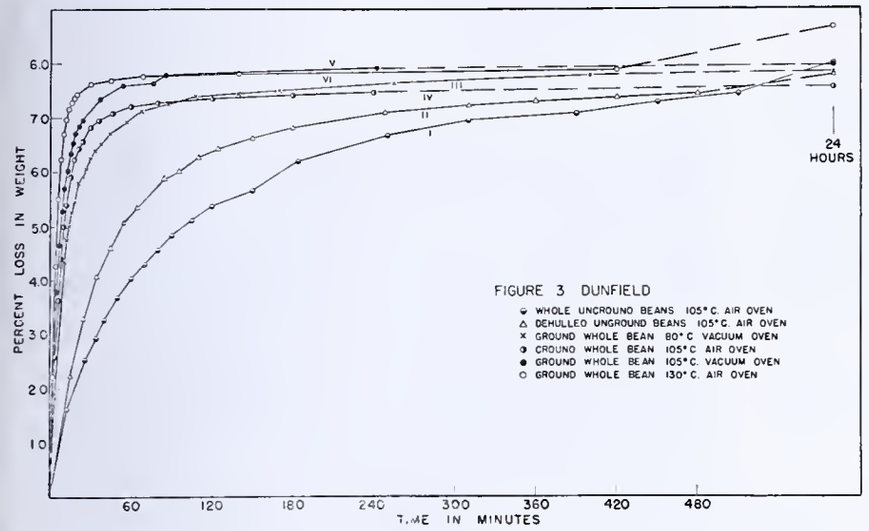
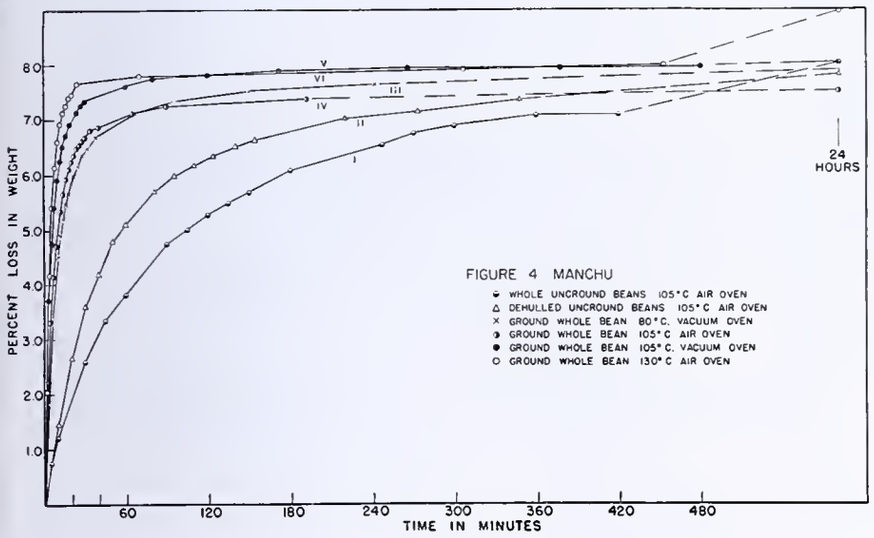


TABLE I. PERCENTAGE OF MOISTURE CALCULATED TO OIL-FREE BASIS[a]

	2 Hours			4 Hours			6 Hours			24 Hours			
	105° C. vacuum	130° C.	Δ	105° C vacuum	130° C.	Δ	105° C. vacuum	130° C.	Δ	105° C. vacuum	Change from 6 hours	130° C.	Change from 6 hours
Illini	9.58	9.59	0.01	9.70	9.75	0.05	9.77	9.86	0.09	9.87	0.10	10.81	0.95
Dunfield	9.80	9.80	0.00	9.91	9.85	0.06	9.91	9.88	0.03	10.00	0.09	10.89	1.01
Manchu	9.71	9.71	0.00	9.86	9.78	0.08	9.90	9.87	0.03	9.98	0.08	11.17	1.30
Giant Green	9.65	9.72	0.07	9.75	9.84	0.09	9.82	9.88	0.06	9.98	0.16	11.12	1.24
Peking	9.72	9.70	0.02	9.85	9.78	0.07	9.85	9.87	0.02	9.99	0.14	10.87	1.00
Variation	0.22	0.21	..	0.21	0.10	..	0.14	0.02	..	0.13	..	0.36	..

[a] A 3-gram sample was used which permitted increments of 0.07 per cent to be determined.


PERCENT LOSS IN WEIGHT
TIME IN MINUTES
24 HOURS
FIGURE 3 DUNFIELD
WHOLE UNGROUND BEANS 105°C. AIR OVEN
DEHULLED UNGROUND BEANS 105°C. AIR OVEN
GROUND WHOLE BEAN 80°C. VACUUM OVEN
GROUND WHOLE BEAN 105°C. AIR OVEN
GROUND WHOLE BEAN 105°C. VACUUM OVEN
GROUND WHOLE BEAN 130°C. AIR OVEN


PERCENT LOSS IN WEIGHT
TIME IN MINUTES
24 HOURS
FIGURE 4 MANCHU
WHOLE UNGROUND BEANS 105°C AIR OVEN
DEHULLED UNGROUND BEANS 105°C AIR OVEN
GROUND WHOLE BEAN 80°C. VACUUM OVEN
GROUND WHOLE BEAN 105°C AIR OVEN
GROUND WHOLE BEAN 105°C. VACUUM OVEN
GROUND WHOLE BEAN 130°C AIR OVEN

in weight by this method are less than those obtained by any of the other methods. They are, in fact, less for 24 hours than the losses observed for 1 hour in the vacuum–105° C. method, for 1 hour at 130° C. in the air oven, or in the vacuum–80° C. method for 3 hours. The only other explanation possible is that the high vacuum as such or the temperature of 130° C. caused the volatilization of some slightly volatile material which would lead to high results. This does not agree with the statement of Cox (*2*) that "considerable work" has shown "that 3 hours at 130° C. in a Freas forced draft oven will produce results agreeing exactly with the toluol method".

The possibility of an error in the loss-in-weight determination, due to a decomposition caused by elevated temperatures or by a combination of atmospheric oxidation and a high-temperature decomposition, must also be considered since it enters into the loss-in-weight value as determined at 130° C. in the air oven. That it does not affect the determination before the 6-hour point seems to be convincingly demonstrated by the essentially identical results obtained by this method and by the vacuum–105° C. method. This identity can be seen in Figures 1 to 5, curves V and VI, and in Table I which gives the values obtained for 2, 4, 6, and 24 hours as well as the differences between the values for the two methods. The agreement of results for 2, 4, and 6 hours is remarkable in that not only was the method different, but the ovens were of different types (the air oven was of the de Khotinsky type,

the several methods, since it has been observed in the Soybean Laboratory on several occasions that samples of very low moisture content were able to remove water from a desiccant such as calcium chloride, when placed over such desiccants in an evacuated desiccator for a period of about 12 hours. Furthermore, it has been observed (*3*) that in the case of cellulosic materials, the dehydrating power in the dry condition is about equal to that of phosphorus pentoxide.

The effect of these humidity conditions and sample behavior would be expected to be most apparent in the case of the moisture determinations in the air oven at 105° C., and such seems to be the case. The losses

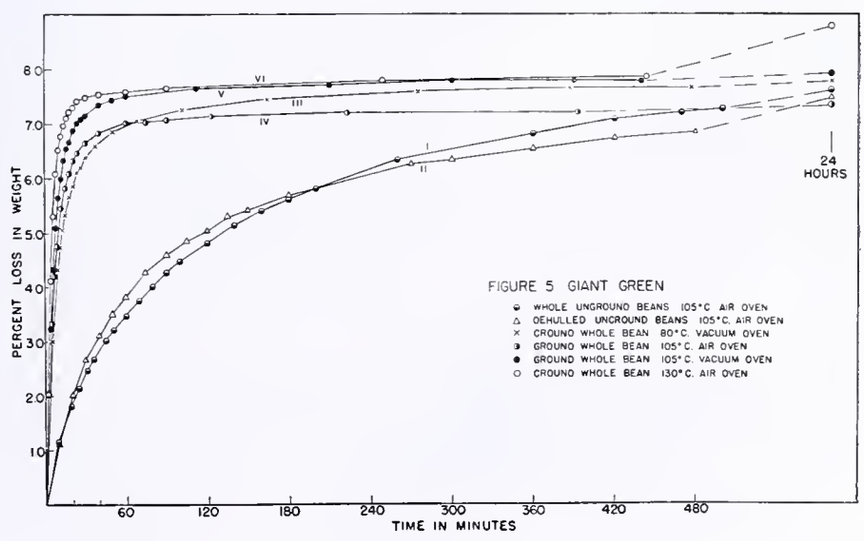

PERCENT LOSS IN WEIGHT
TIME IN MINUTES
24 HOURS
FIGURE 5 GIANT GREEN
WHOLE UNGROUND BEANS 105°C AIR OVEN
DEHULLED UNGROUND BEANS 105°C. AIR OVEN
GROUND WHOLE BEAN 80°C. VACUUM OVEN
GROUND WHOLE BEAN 105°C. AIR OVEN
GROUND WHOLE BEAN 105°C. VACUUM OVEN
GROUND WHOLE BEAN 130°C. AIR OVEN

Table II. Nitrogen, Oil, and Moisture Contents

	Nitrogen			Moisture[b] (Ground Whole Bean) 130° C., 20 Minutes
	Whole basis	Oil-free basis	Oil	
	%	%	%	%
Peking[a]	6.14	7.30	15.92	7.80
Giant Green	5.89	7.43	20.79	7.35
Illini	5.83	7.26	19.66	7.33
Manchu	6.08	7.56	19.61	7.40
Dunfield	5.80	7.30	20.52	7.50

[a] Moisture determined by usual laboratory method: 3 hours, 130° C., 8.22; 3 hours, 105° C., 7.80.
[b] Determined by usual laboratory method.

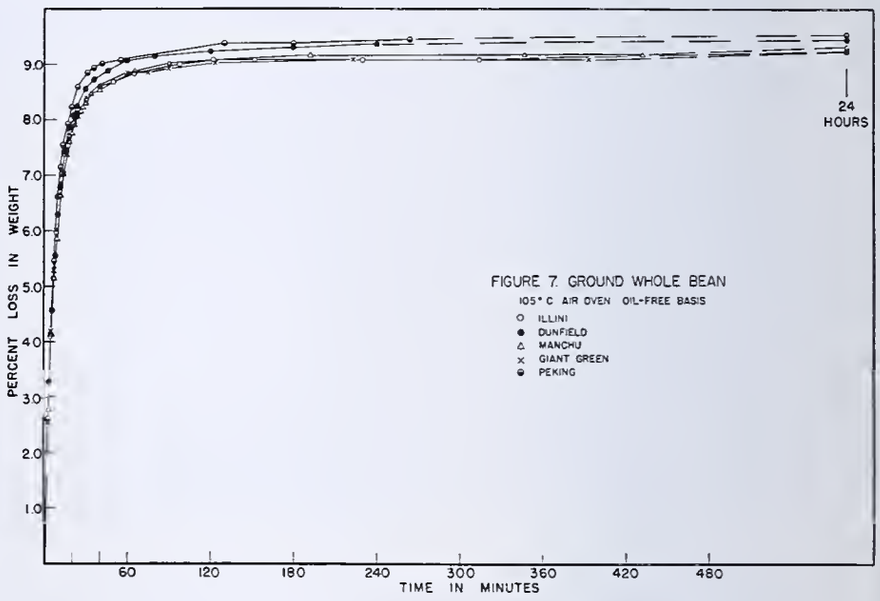
and the vacuum oven was of the oil-jacketed type), and the measuring devices were separate units. In the case of the Manchu bean the 130° C. determination was allowed to continue until for the 72-hour period the loss of 12.52 per cent indicated that the loss due to decomposition was continuing.

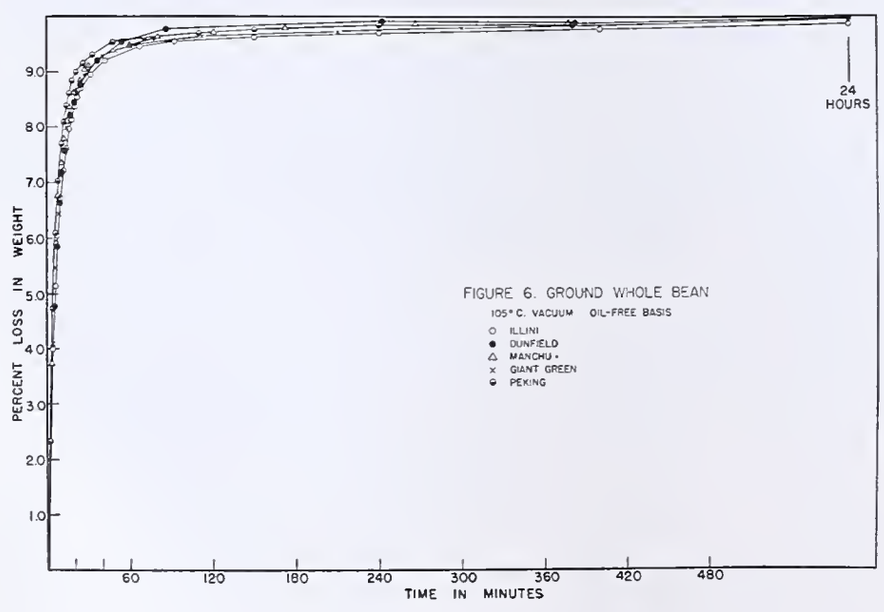
The very small loss occurring during the final 18-hour period of the vacuum–105° C. method (Table I) indicates the reliability of the 24-hour value. The Dunfield sample was allowed to continue for an additional 24-hour period, during which there was a loss of only 0.07 per cent.

All the values for moisture reported in Table II were obtained by the usual laboratory dish method and are the average of two determinations. The 130° C. values were obtained in a Freas circulating air oven and the 105° C. value in a de Khotinsky air oven. The significant point about these values is that they fall exactly on the loss-in-weight curves obtained with the loss-in-weight apparatus in the other ovens. This indicates that the results obtained are identical at 130° C. in an air oven, 130° C. in a forced draft oven, and 105° C. under a vacuum in an oil-jacketed vacuum oven. It is impossible to correlate the results obtained at 105° C. in an air oven with those obtained by the other methods.

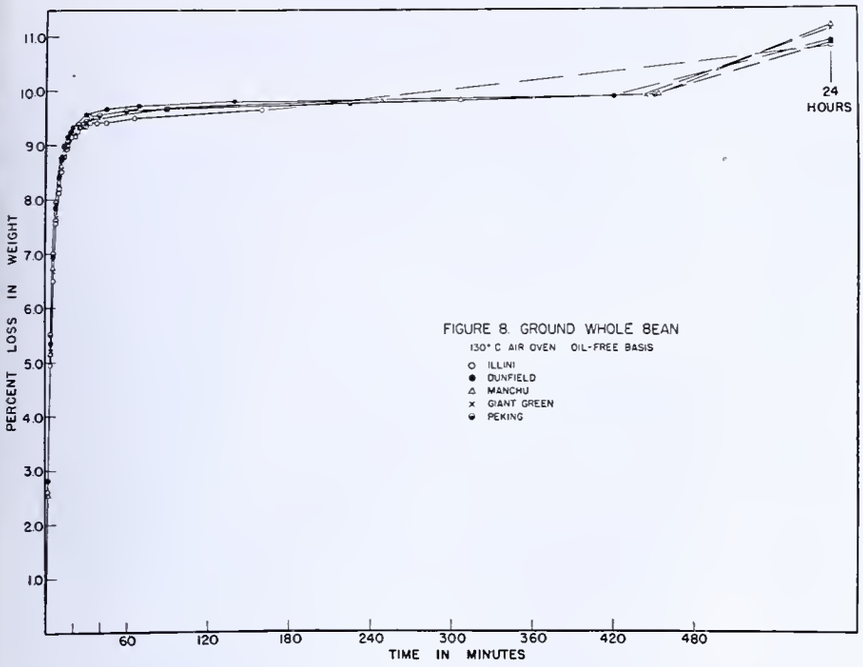
In regard to the small but fairly consistent variation in the moisture content of the several samples of beans, the sample having the lowest nitrogen content on the oil-free basis also has the lowest moisture content at every point on the curves. In view of the small difference in nitrogen content (0.30 per cent on the oil-free basis) it is not safe to assume that all carefully conditioned samples would fall within the narrow range of moisture values found in this study. An investigation of a series of samples having a range of nitrogen content is in progress at the Soybean Laboratory.

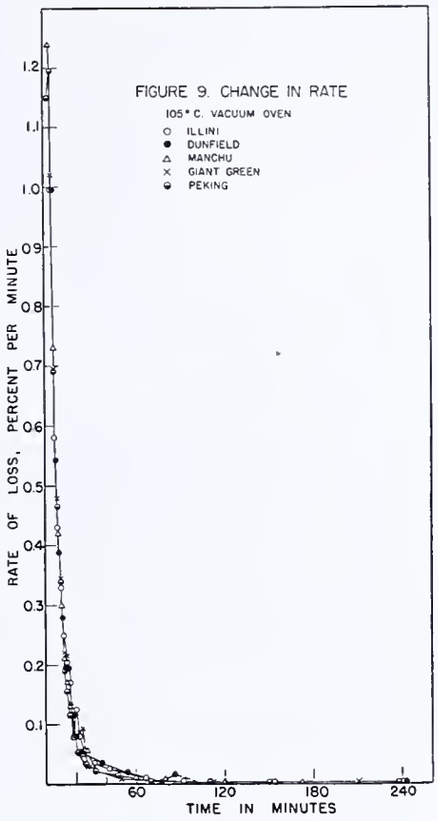
In ascertaining a practical length of time for the moisture determination, it is essential that the analyst be given some latitude as to the moment for removing the samples from the oven. With this point in view, the rate of loss was calculated and plotted against the time at which such rates were prevailing (Figure 9). If a consistency of results of less than 0.10 per cent is desired, it is clear that the rate of loss must be less than 0.01 per cent per minute if the interval for removal is limited to 10 minutes or 0.005 per cent per minute if the interval is extended to 20 minutes. For the 105° C.–vacuum method the above conditions are found when the determination has proceeded beyond the 90- and 100-minute periods, respectively, and for the 130° C. method, beyond the 75- and 90-minute periods. For values of the moisture content which are to be used for the correction of other analytical data to the moisture-free basis or otherwise, it is apparent from Figures 6 and 8 that consistent results will be obtained by selecting for the complete analysis any length of time from 1.5 to 8 hours for the 130° C. method or from 1.5 to 24 hours for the 105° C.–vacuum method. Furthermore, the variation in the results during these periods would be insufficient to produce a significant error when used in corrective calculations.





The rate curves brought to light the singular phenomenon that the change in rate is not a smooth deceleration as it would appear from a glance at the loss-in-weight curves (Figures 1 to 8) but that abrupt changes take place, often with a short period of acceleration. The fact that about 50 per cent of the observed breaks were duplicated when the sample was analyzed under different conditions and that at some other points several varieties checked each other, eliminated the possibility of chance errors in observation. For example, the 105° C.–vacuum method showed transitions for the Illini and Giant Green varieties at 8.96 and 8.95 per cent, respectively, and the 130° C. method showed transitions for the same varieties at 8.94 and 8.96 per cent. This observation is now being investigated more fully.





Unground Beans and Permeability of Hulls

In studying the relationship of curves I and II (Figures 1 and 5) which represent, respectively, the whole unground bean and the unground bean with the hull removed, it is evident that a number of factors must be considered. Factors contributing to the difference in the curves might be considered to be: (1) permeability of the seed coat, (2) fragility of the seed coat, and (3) size of the bean. The first two factors must be considered together, since in only one case, that of the Peking variety, was the seed coat intact at the end of the experiment.

The curves for any single variety express the difference in percentage loss which is caused by the hull, since all other factors are identical. The greatest separation is to be noted in the case of the Peking bean (Figure 1) and the least in the case of the Giant Green (Figure 5).

If the rate of diffusion of the moisture through the bean itself was considerably less than the rate through the hull, the curves would lie very close together. Since they do not lie close together in all cases, it is of some importance to observe that the character of the hull is a factor in determining the rate at which a given variety of bean loses or gains moisture.

The actual permeability has been investigated in two cases—the Giant Green and the Peking. These varieties were chosen because they showed the most divergent effect due to the presence of the hulls as well as the greatest difference in the average size of the beans. The average weight of the Giant Green was 0.304 gram per bean while that of the Peking was 0.079 gram.

The area of the beans was determined by quantitatively removing the hulls from a definite number and weight of the beans, flattening the hulls between glass plates or celluloid sheets, tracing the image as enlarged in an enlarging camera, and then determining the area of the enlargement with a planimeter. Dividing the value for this area by the enlargement factor gave the actual surface area of the beans. The area of the Giant Green was 5.75 sq. cm. per gram and that of the Peking was 9.41 sq. cm. per gram. These values permitted the calculation of the loss in weight per square centimeter per minute.

The rate per unit area was constant in the case of the Peking variety in the interval from 4.5 to more than 6.0 per cent loss in weight on the oil-free basis, and for the Giant Green from 4.0 to 5.5 per cent loss in weight. This constant rate, and the fact that the vapor pressure at the outside surface of the hull is very low and constant at the temperature of the vacuum oven, indicate that the driving force at the inside surface of the hulls was constant. That the forces in the two varieties are equal would seem probable in view of the fact that both varieties have very nearly the same percentage of moisture and nitrogen. If this is true, then a comparison of the rates per unit area under these conditions is in fact a comparison of the permeabilities. The rate per square centimeter for the Peking is 0.0011 per cent per minute and for the Giant Green is 0.0020 per cent per minute. The ratio indicates that the hull of the Giant Green is 1.8 times as permeable as that of the Peking. This method of determining permeabilities may be of value in studies relating to the germination of beans.

Beyond a total loss of about 5.5 per cent, the rates per unit area for the two curves representing the Giant Green were identical, indicating that at this point the hulls began cracking.

In general, curve I (Figures 1 to 5), representing the percentage loss in weight of whole beans, combines the effects of the several factors and is of interest in comparing the relative changes in moisture content of beans in quantity.

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The U. S. Regional Soybean Industrial Products Laboratory is a cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Station of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

Correction. In the article on "Spectrophotometric Methods in Modern Analytical Chemistry" [Ind. Eng. Chem., Anal. Ed., **11**, 75 (1939)] there was an error in placing the decimal point on the extinction coefficient of copper diethyldithiocarbamate in Table II. $k\lambda(E)$ should read 5310. and not 531. The second sentence below Table II should read: "Actually (Table II) it is about ten times as good."

S. E. Q. Ashley

Labile Sulfur

The Use of Thallous Nitrate

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THE similarity in chemical behavior of lead and thallous salts suggested the possible use of thallous nitrate for the qualitative and quantitative determination of labile sulfur in proteins.

Early investigators [Fleitmann (*7*), Krüger (*8*), Osborne (*13*), and Mörner (*12*)] found that all the sulfur of the proteins could not be liberated as sulfide sulfur on boiling the samples with strong alkali and that the values obtained varied within wide limits, depending upon experimental conditions and the protein under investigation. Mörner (*12*), describing a method for the determination of *bleischwärzender Schwefel* (lead-blackening sulfur), compared the values obtained by the methods of Fleitmann (*7*), Malerba (*9*), Middledorf (*11*), Schulz (*14*), and Osborne (*13*). Maxwell, Bischoff, and Blatherwick (*10*) reported a micromethod for the determination of labile and total sulfur in proteins. Sheppard and Hudson (*15*) developed a colorimetric method for the determination of labile sulfur in gelatin and proteins depending on the blue color produced with *p*-aminodimethylaniline in the presence of ferric chloride. Zahnd and Clarke (*16*) devised a method for the quantitative determination of labile sulfur under conditions suitable for the estimation of small amounts of cystine in cystine solutions and protein hydrolyzates. In a recent paper, Blumenthal and Clarke (*3*) described a modification of the alkaline plumbite method.

In preliminary qualitative tests in which the alkaline plumbite method was replaced by an alkaline solution of thallous nitrate, it was found that solutions of cysteine, cystine, and sulfur-containing proteins gave a precipitate of thallous sulfide. This qualitative test proved to be at least as sensitive if not more so than the conventional plumbite method. It was next decided to study the use of thallous nitrate in an alkaline medium for the quantitative determination of labile sulfur and to compare this action with that of the plumbite procedure. Accordingly, an investigation was undertaken to determine whether the thallous ion would quantitatively remove the labile sulfur from cystine and cysteine, and to compare the rate of removal of the sulfur from these two amino acids using both the thallous and plumbite procedures. Furthermore, several proteins were investigated with respect to the rate of formation of thallous sulfide by the action of thallous nitrate in a strongly alkaline medium. In the course of the work, a technique was developed for a practical working method when using thallous nitrate.

Experimental

QUALITATIVE DETERMINATION. To 2 cc. of a 20 per cent solution of sodium hydroxide, add a small amount of the substance to be examined. Heat to boiling, then add 3 drops of a saturated solution of thallous nitrate. Labile sulfur is indicated by the darkening of the solution or the appearance of a black color on the surface of the suspended material. If the substance upon examination is suspected to be low in labile sulfur, permit the solution or suspension to stand for several minutes, as the appearance of the black color may develop slowly.

In addition to the proteins investigated quantitatively, blood albumin, edestin, globulin, and zein gave positive results when subjected to this qualitative examination.

QUANTITATIVE DETERMINATION. Dissolve small quantities of cysteine hydrochloride, ranging from 23.1 to 32.9 mg., in 5 cc. of a 1 *N* hydrochloric acid solution, add 50 cc. of a 20 per cent solution of sodium hydroxide and 10 cc. of a 0.4 *N* solution of thallous nitrate, and reflux the solution for known intervals of time. At the end of these periods separate the thallous sulfide formed by centrifuging and oxidize it according to the method of Zahnd and Clarke (*16*). After complete oxidation, when all the sulfur is dissolved, gradually warm the mixture on a water bath until there is no further evolution of chlorine. Then cool the solution and add a 20 per cent solution of sodium hydroxide in slight excess of the amount necessary to precipitate the reddish-brown thallic oxide. Separate the thallic oxide by centrifuging and transfer the supernatant liquid to a small beaker. In this process, most of the excess thallic ion is removed in order to prevent its interfering seriously with the subsequent precipitation of the sulfate as barium sulfate.

Adjust the pH of the solution to the optimum for barium sulfate precipitation, the final volume of the solution being about 100 cc. Precipitate the sulfate quantitatively with barium chloride, filter it off, wash, and ignite. The results are very inconsistent and much above the expected recovery. Treat the ash with 4 to 5 drops of concentrated sulfuric acid, heat the whole very carefully on an air bath, and subsequently heat to constant weight. A distinct increase in weight is noted. Wash the ash with small quantities of hot water, using a porcelain spatula to break up the small particles of barium sulfate, filter, and ignite. A loss in weight is noted. After repeating the sulfuric acid treatment, no further gain or loss in weight is observed.

Follow the same procedure, substituting solutions of cystine for the cysteine hydrochloride, 5 cc. of the cystine solution being equivalent to 20 mg. of cystine.

The amount of protein used in the determinations is dependent upon the per cent of labile sulfur expected (Table I). To the dried sample add 50 cc. of a 20 per cent solution of sodium hydroxide and heat until solution of the protein takes place. Add 10 cc. of a 0.4 *N* solution of thallous nitrate, and reflux the sample for known intervals of time in order to determine the rate at which the thallous sulfide is formed and to ensure maximum recovery. The rest of the procedure is identical with that followed for cysteine and cystine.

In Table I are indicated the maximum labile sulfur values obtained for cysteine, cystine, and commercial grades of casein, egg albumin, fibrin, pepsin, and trypsin.

TABLE I. LABILE SULFUR IN BIOLOGICAL SUBSTANCES

Substance	Weight of Substance	Weight of $BaSO_4$	Labile S
	Mg.	*Mg.*	%
Cysteine.HCl	32.9	46.3	95.09
	24.5	34.1	93.98
	25.0	35.1	94.69
			Av. 94.59
Cystine[a]	20.6	30.9	77.80
	20.6	30.9	77.80
			Av. 77.80
Casein (Eimer & Amend, pure)	5000	34.1	0.094
	5000	31.8	0.087
	5000	33.0	0.091
			Av. 0.091
Egg albumin (Merck, impalpable powder)	1000	44.4	0.610
	1000	44.2	0.607
			Av. 0.609
Fibrin (Eimer & Amend, pure)	1000	34.3	0.471
	1100	38.2	0.476
	1330	47.5	0.490
			Av. 0.479
Pepsin (Eimer & Amend, U. S. P.)	1000	18.8	0.258
	1000	18.8	0.258
	1000	19.0	0.261
			Av. 0.259
Trypsin (Eimer & Amend, pure)	1000	28.8	0.395
	1000	30.8	0.423
	1000	29.0	0.398
			Av. 0.405

[a] Weight of cystine in each case is 20.6 mg. in 5 cc. of stock solution.

In Table II are reported the rates of formation of the labile sulfur. Each value represents the average of three individual determinations.

In Figure 1 the action of thallous nitrate on cysteine hydrochloride and on cystine is compared with that of lead nitrate. The concentrations of the lead nitrate and alkali are identical with that used in the case of the thallous nitrate procedure. Each point plotted represents the average value of three determinations.

Discussion

The qualitative procedure described for labile sulfur proved to be at least as sensitive as the conventional lead method. Furthermore, it is felt that sufficient quantitative results of good reproducibility have been obtained to permit the use of the new quantitative technique reported.

An examination of Figure 1 indicates that under the same experimental conditions the action of thallous and lead ions on cystine and cysteine is essentially the same. Cystine yields but three fourths of its sulfur as labile sulfur, whereas practically all of the sulfur of cysteine can be recovered in this form. These observations are in agreement with the results of Clarke and Inouye (*5*).

Bergmann and Stather (*2*) as well as Brand and Sandburg (*4*) found that the lability of the sulfur atom towards alkali differs enormously in the different compounds of cystine. Furthermore, Abel and Geiling (*1*) and du Vigneaud (*6*) showed that when cystine is a constitutent of the insulin molecule, the ease of lability is enhanced considerably as compared with cystine itself. An examination of the results reported in Table II indicates this increased sulfur lability of the cystine and cysteine sulfur when these are structural units of the protein molecule.

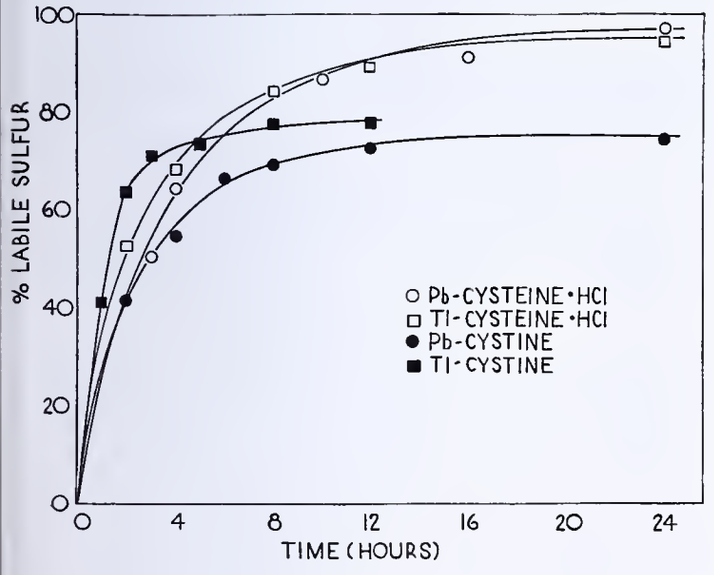

Figure 1. Action of Thallous and Lead Nitrate

Finally, the labile sulfur values of the proteins were obtained by the direct action on the commercial grades, as indicated in Tables I and II, rather than on the crystalline products. As such, the reported labile sulfur values of the proteins are primarily important in that they are illustrative of the reproducibility of the results. Nevertheless the values for casein and fibrin are in close agreement with the values reported by Zahnd and Clarke (*16*). The value for egg albumin is much higher and must be ascribed to the differences in the commercial product examined (*16*). The values for pepsin and trypsin could not be found in the literature.

Table II. Action of Alkaline Solution of Thallous Nitrate on Biological Substances

Substance	Refluxing *Hours*[a]	Labile S %
Cysteine.HCl	2	52.75
	4	68.21
	8	84.38
	12	89.10
	24	94.59
Cystine	1	40.92
	2	63.91
	3	71.10
	5	73.89
	8	77.55
	12	77.80
Casein (Eimer & Amend, pure)	1	0.075
	2	0.083
	3	0.089
	6	0.091
Egg albumin (Merck, impalpable powder)	0.5	0.483
	1	0.608
	4	0.596
	6.5	0.609
Fibrin (Eimer & Amend, pure)	1	0.479
	3	0.474
	6	0.467
Pepsin (Eimer & Amend, U. S. P.)	2	0.224
	4	0.259
	6	0.255
Trypsin (Eimer & Amend, pure)	2	0.355
	4	0.391
	6	0.405

[a] Does not include time necessary to dissolve samples.

Conclusions

1. Methods for the qualitative and quantitative determination of labile sulfur in biological substances have been devised.

2. The action of lead and thallous ions in alkaline medium on cystine or cysteine is essentially the same, cystine yielding but three fourths of its sulfur, whereas cysteine yields all of its sulfur in the labile form.

3. The labile sulfur values as determined by the thallous nitrate procedure of casein (Eimer & Amend pure), egg albumin (Merck, impalpable powder), fibrin (Eimer & Amend, pure), pepsin (Eimer & Amend, U. S. P.), and trypsin (Eimer & Amend, pure) are, respectively, 0.091, 0.609, 0.479, 0.259, and 0.405 per cent.

Acknowledgment

Some years ago, the possibility of using thallous nitrate for the qualitative and quantitative estimation of labile sulfur was suggested to the senior author by H. T. Clarke of the College of Physicians and Surgeons, Columbia University, New York, N. Y.

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Estimation of Gossypol in Crude Cottonseed Oil

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A clear-cut method for the estimation of gossypol in crude cottonseed oil is presented, with modifications which permit sets of ten determinations to be made fairly expeditiously. Precipitation is expedited by increased temperature, by the addition of gossypol in an ether-extracted oil prepared from cottonseed meats, and by constant agitation which precipitates the gossypol in a good crystalline condition for rapid filtration and washing without appreciable loss due to dissolving. Solubility is prevented by the use of pyridine in the wash solution. The gossypol compound is prevented from adhering to the glass container by the elimination of practically all water. Recovery of added gossypol and the reproducibility of results are good.

THE observation of Royce (*2*) that pyridine promotes the precipitation of gossypol from crude cottonseed oil has been confirmed. However, Royce's method of estimating gossypol in crude cottonseed oil (*2*, *3*) can be improved in a number of respects. Gossypol in the presence of pyridine is not precipitated as dianilinogossypol but as dianilinogossypol dipyridine, a salt of gossypol (*5*). Driving off all the pyridine of crystallization by heating the precipitate at 100° C. to constant weight, in the authors' hands, has not given consistent results and has not been practical. The factor 0.775 for converting dianilinogossypol to gossypol is not applicable unless all the pyridine has been removed.

The process of precipitating gossypol from crude oil is slow and tedious and the precipitate at times adheres tenaciously to the glass vessel in which precipitation occurs. The dianilinogossypol containing pyridine of crystallization is appreciably soluble in the petroleum ether used in washing, in contrast to the relatively insoluble dianilinogossypol alone. A rapid clear-cut method is desirable. The precipitation of gossypol should be fairly rapid and complete. It should be readily removed from the flask in which precipitation occurred and washed without appreciable loss.

In attempting to overcome these objections the following factors were studied: the charge of crude oil necessary to expedite the precipitation of gossypol; the advantage of adding gossypol to the charge of crude oil in order to expedite precipitation; the quantity of reagents necessary; the elimination of water from the reagents used in the precipitation of gossypol; the temperature and agitation necessary for a more rapid and complete precipitation; the prevention of loss of the precipitated gossypol by washing; and the length of time necessary for heating the precipitate to constant weight by driving off all the pyridine.

Method of Determination

Filter the crude cottonseed oil by gravity through glass wool. Weigh a charge of 25 grams of crude oil into a 250-ml. Erlenmeyer flask and pipet 5 ml. of cottonseed oil containing considerable gossypol (extracted from cottonseed meats with ethyl ether) into the same Erlenmeyer flask, wiping off all the oil on the outside of the filled pipet with a clean cloth before adjusting the oil to the mark. After emptying the contents of the pipet into the flask, wash oil adhering on the inside into the flask with petroleum ether (boiling point 30° to 60° C.). Into another Erlenmeyer flask containing 25 grams of refined cottonseed oil (Wesson) pipet 5 ml. of ether-extracted oil from cottonseed meats to determine the amount of gossypol added to the unknown charge. To each charge add 75 ml. of petroleum ether (boiling point 30° to 60° C.) and after allowing to stand overnight in the lightly stoppered flasks, add about 0.1 gram of Hyflo-Supercel to the mixture in each flask. Filter off the precipitated material with suction through dry Gooch crucibles (which contain a mat of Hyflo-Supercel over the asbestos pad) into a 250-ml. Erlenmeyer flask placed under a bell jar. Wash the flask and precipitate in each Gooch crucible with a small quantity of petroleum ether (10 to 15 ml.).

Add the following reagents per determination: 4 ml. of aniline and 12 ml. of fractionated pyridine (boiling point 115° to 116.3° C.). Agitate 72 hours in a shaking constant-temperature water bath held at 43° to 46° C., using water-cooled condensers (Hopkins reflux type) attached to the Erlenmeyer flasks.

In order to catch the few drops of water which form in the condenser tube, attach a small glass cup to the lower end of the cork stopper through which the condenser tube passes. The elimination of water prevents the precipitate from adhering to the glass vessel.

After 72 hours' shaking at 43° C. and 1 hour's standing out of the bath, filter with suction through a tared Gooch crucible, first decanting off a portion of the mother liquor into a 150-ml. Erlenmeyer flask for use in washing out any precipitate which adheres to the flask. The precipitate is conveniently washed from the Erlenmeyer flask with the aid of a special wash bottle (*4*) containing petroleum ether and dry pyridine (100 + 3). Loosen any precipitate which adheres to the flask, with the aid of a rubber-tipped glass rod and the mother liquor. After washing down the walls of the Gooch crucible, wash the precipitate five times by filling the crucible about half full of the petroleum ether–pyridine mixture, using suction (about 25.4 cm., 10 inches). Finally wash down the sides of the Gooch crucible carefully with the petroleum ether–pyridine mixture and wipe the outside surface clean with a cloth.

Dry the precipitate in a hot air oven at 110° C. (by heating 18 to 24 hours to constant weight, depending upon the amount of precipitate) in order to drive off the two molecules of pyridine.

TABLE I. RECOVERY OF PURIFIED GOSSYPOL FROM REFINED OIL AND FROM ETHER-EXTRACTED OIL

Gossypol Charge *Mg.*	Ether-Extracted Oil (Meats) *Ml.*	Total Dianilinogossypol *Mg.*	Gossypol Recovered[a] *Mg.*
	Purified Gossypol in Refined Oil		
100	..	128.1	
100	..	128.1	
		Av. 128.1	99.3
100	..	127.3	
100	..	127.3	
100	..	128.7	
		Av. 127.8	99.1
	Gossypol in Ether-Extracted Oil (Meats)		
...	5	133.6	
...	5	134.6	
...	5	134.8	
...	5	135.1	
		Av. 134.5	104.2
...	5	133.3	
...	5	132.2	
		Av. 132.8	102.9
	Purified Gossypol Added to Ether-Extracted Oil (Meats)		
50	5	200.1	
50	5	200.4	
50	5	199.8	
50	5	199.9	
		Av. 200.1	155.0
50	5	198.4	
50	5	198.0	
50	5	199.5	
50	5	199.2	
		Av. 198.8	154.1

[a] 0.775 × weight of dianilinogossypol.

The total weight of dianilinogossypol in the charge, less that added in the 5 ml. of ether-extracted cottonseed oil, multiplied by 0.775, gives the equivalent amount of gossypol. Four times this amount of gossypol gives the per cent of gossypol present in crude oil.

Reagents Used

Gossypol Added to Charge. An ether-extracted oil relatively high in gossypol was used, which was obtained by the extraction of prime cottonseed meats with peroxide-free ether. The meats consisted of the flattened decorticated seed passed through iron rolls set to within 0.025 mm. (0.001 inch), and were taken just before they entered the steam cookers (or the press in the expeller process).

The ethyl ether was removed from the extracted oil by distillation, by placing the distilling flask in a water bath heated by a closed steam coil in which the temperature of the water was not allowed to rise above 60° C. The hot oil was filtered through glass wool and any residual ether removed under reduced pressure while the flask was gently warmed in a vessel of warm water.

The ether-extracted oil was stored at about 5° C. in an electric refrigerator. For the determination of its gossypol content, 5 ml. charges were taken after the oil had reached a temperature of 25° to 26° C. and had been carefully mixed by shaking.

Petroleum Ether (boiling point 30° to 60° C.), Skellysolve F, and aniline (from sulfate or practical), Eastman.

Pyridine (boiling point 115° to 116.3° C.) was obtained by the fractionation of pyridine (practical, 112° to 117° C.), Eastman, in order to eliminate water. All grades examined contained water, which, if present in excessive amounts, may interfere with the precipitation of gossypol.

Table II. Reproducibility of Results on Crude Cottonseed Oil (No. 2549)

Charge	Ether-Extracted Oil (Meats)	Dianilinogossypol	Less amount added[a]	Gossypol	
Grams	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*%*
25	5	154.8	22.2	17.2	0.069
25	5	155.3	22.7	17.6	0.070
25	5	154.3	21.7	16.8	0.067
					Av. 0.069
..	5	132.5	..	..	...
..	5	132.5	..	..	...
..	5	132.9	..	..	...
		Av. 132.6			
25	5	154.1	21.3	16.5	0.066
25	5	154.2	21.4	16.6	0.066
25	5	153.1	20.3	15.7	0.063
					Av. 0.065
..	5	132.6	..	..	...
..	5	133.0	..	..	...
..	5	132.7	..	..	...
		Av. 132.8			

[a] From ether-extracted oil (meats).

Accuracy of Method

The accuracy of the method is indicated by the quantity of gossypol recovered (99.3 and 99.1 mg.) from 100 mg. of purified gossypol dissolved in 25 grams of Wesson oil (Table I); from 50 mg. of purified gossypol added to 5 ml. of the ether-extracted oil mixed with 25 grams of refined oil, and from 5 ml. of the extracted oil mixed with 25 grams of refined oil. When the quantity of gossypol precipitated from 5 ml. of extracted oil is deducted from that precipitated in 5 ml. of extracted oil plus 50 mg. of added purified gossypol the result, 50.8 and 51.2 mg., shows that the added purified gossypol was recovered.

The 50 mg. of gossypol added to the 5 ml. of ether-extracted oil promotes the precipitation of gossypol from the latter, and it is probable that at least a portion of the gossypol figured as recovered from the 50-mg. charge is in reality additional gossypol precipitated from the ether-extracted oil. The addition, in the ether-extracted oil of a relatively large amount of gossypol (approximately 100 mg.) also promotes the precipitation of additional gossypol from the crude oil. This slightly increased recovery is not accounted for by the occurrence of small amounts of a phosphorus-containing compound in the precipitated dianilinogossypol.

Compilation of previous results on dianilinogossypol obtained from 5 ml. of the ether-extracted oil gave a mean of 133.72 ± 1.38 mg. which is equivalent to 103.63 ± 1.07 mg. of gossypol (59 determinations).

From refined cottonseed oil 99.3 and 99.1 per cent of 100 mg. of purified gossypol was recovered. Solution in the reagents probably accounts for the slightly low results.

Reproducibility of Results

Representative results (Table II) from a crude oil containing an average amount of gossypol show the usual variations obtained. The averages of 3 determinations may differ by 0.004 to 0.006 per cent.

Discussion of Factors Studied

The detailed data upon which the amounts of reagents recommended are based are not presented here. Tables I and II, in which these amounts of reagents were used, show the percentage recovery and reproducibility of results.

Gossypol is more readily recovered from refined cottonseed oil than from the commercial crude oil, and can be more readily precipitated from an oil extracted by ether from the decorticated cottonseed or meats.

The commercial crude oils usually contain from 15 to 40 mg. of gossypol in a 25-gram charge. These small amounts are more readily and completely precipitated by the addition of about 100 mg. of purified gossypol or approximately that amount in 5 ml. of the ether-extracted oil from the meats.

The gossypol content of crude oils, even though kept in cold storage, decreases over a period of months.

With the amounts of reagents and solvent kept the same a charge of 25, instead of the 50 grams recommended by Royce, gave slightly higher yields of gossypol. The precipitation of gossypol was expedited by adding to the charge 5 ml. of an ether-extracted oil from cottonseed, which had a high gossypol content (approximately 100 mg. in 5 ml.). The use of the ether-extracted oil was more convenient than the longer process of purifying gossypol for this purpose, as the latter does not keep well at ordinary laboratory temperature. Adding gossypol to the unknown charge gave a more complete and rapid precipitation, especially where the amount of gossypol in the oil was small (*3*).

The procedure given above—filtering off a flocculent precipitate from the oil–petroleum ether mixture after it had stood overnight—probably does not remove all this material.

Raymond Reiser found by the Fiske and Subbarow method that the weighed precipitate of dianilinogossypol contained a small amount of phosphorus (0.62 mg. or 0.4 per cent) which was probably due to the phosphatides lecithin and cephalin (*1*). The amount of this contaminant was not significant, as the nitrogen content of the dianilinogossypol checked theory.

All grades of pyridine examined contained water, in some instances in rather large amounts and this had to be removed by fractional distillation, using the portion having a boiling point of 115° to 116.3° C. The reaction of aniline with gossypol also yields a small amount of water. The few condensed drops of water were prevented from coming in contact with the dianilinogossypol dipyridine precipitate in the bottom of the Erlenmeyer flask by being caught in a small cup attached directly beneath the condenser tube. The cup was made by cutting off a 20-mm. test tube about 1 cm. from the bottom, and sealing to the bottom section a small glass rod about 3 cm. long, one end of which was drawn to a sharp point. This end was stuck into the lower surface of the cork stopper through which the condenser tube passes. A small amount of precipitate occasionally adhered to the glass container, indicating that a small quantity of moisture probably remained.

Loss of precipitate due to solubility was prevented by using a decanted portion of the mother liquor in transferring the precipitate to the Gooch crucible and by washing with minimum amounts of petroleum ether containing 3 parts of the fractionated pyridine per 100 ml. of petroleum ether (boiling point 30° to 60° C.). Heating the precipitate in an air oven to constant weight at 110° C. drove off all the pyridine. The nitrogen content of the heated residue showed it to be dianilinogossypol (*5*).

Need for Constant Temperature and Agitation

Considerable difficulty was encountered in obtaining complete precipitation of the last 1 to 3 mg. of gossypol. In order to expedite and complete the precipitation, constant agitation with increased temperature was found necessary. The production of fair-sized crystals and the most complete precipitation of gossypol occurred with moderate shaking at 43° to 46° C.

The crystals of precipitated gossypol should be large enough so that they will not pass through the Gooch crucible or clog it while filtering. Too quick precipitation of the gossypol compound results in small crystals, and too vigorous shaking causes a fine precipitate to form rapidly. If the solution is not sufficiently agitated, the gossypol precipitation may not be complete in 72 hours. When shaken 48 hours, 97 to 98 per cent of the gossypol was recovered. An additional 24 hours of shaking was necessary for a more complete precipitation. Precipitation also requires proper control of the temperature.

Agitation was accomplished in a constant-temperature water bath suspended by 20.3-cm. (8-inch) arms from center to center from a wood frame by bearings. The bath holding 10 Erlenmeyer flasks is moved back and forth with a stroke of 1.27 cm. (0.5 inch), approximately 120 revolutions per minute, by means of an electric motor with the proper reducing gears (Eimer and Amend, ball mill, No. 21,481/1) and with an offset of 0.64 cm. (0.25 inch) off center for the connecting arm on the reducing gear to the water bath. The water bath had the following outside and inside dimensions: 40.6 × 87.7 × 24.1 cm. (16 × 34.5 × 9.5 inches) and 33.0 × 81.3 × 20.3 cm. (13 × 32 × 8 inches), respectively. The bath was lined with 28.35-gram (16-ounce) soft sheet copper and was surrounded with a layer of plywood (Celotex) made from 24.1 × 19.1 cm. (9.5 × 0.75 inch) boards.

Two heating units made from No. 25 Chromel A wire were drawn through two 2.54-cm. (1-inch) copper pipes lined with asbestos paper except at the top surface. They were placed 1.27 cm. (0.5 inch) from the bottom of the bath, running lengthwise through it. The Chromel A wire was 43.7 and 38.1 cm. (17.2 and 15 feet) long, having 25.3 and 30.5 ohms resistance, giving 3.16 and 3.65 amperes with a wattage of 347 and 402, respectively.

Summary

A comparatively rapid method for the estimation of gossypol in crude cottonseed oil is presented. The gossypol was precipitated in a good crystalline condition at 43° C. by constant agitation for 72 hours. Precipitation was in part expedited by the addition of gossypol in an ether-extracted oil made from cottonseed meats. The crystalline precipitate was readily washed and filtered. The gossypol compound was prevented from adhering to the glass container in which precipitation occurred by the elimination of practically all water. Loss in washing the precipitate was also largely prevented.

A rather high recovery of gossypol was obtained with good reproducibility of results.

Acknowledgment

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Rapid Method for Determination of Manganese in Feeds

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METHODS for the determination of manganese have received much attention in the past due to the importance of manganese in both the steel industry and agriculture. Much more interest has been shown in the determination of manganese in biological materials since Wilgus, *et al.* (*10*) showed that this element is important in the prevention of perosis in chicks. Most methods that are used for the preparation of a sample previous to the determination of manganese in biological materials involve a lengthy dry-ash procedure which requires a number of steps of manipulation. Also, it was shown by Bolin (*2*), Davidson (*3*), and others that the acid-insoluble portion of ash contained appreciable quantities of manganese, but that it could be recovered by a sodium carbonate fusion or by volatilization of silica with hydrofluoric acid.

Hundreds of samples of feeding materials have been wet-ashed in this laboratory by the procedure outlined by Gerritz (*4*), using a nitric acid oxidation followed by a perchloric acid oxidation previous to the determination of calcium and phosphorus. This procedure is rapid and lends itself well to the analysis of feed ingredients and plant materials. It seemed desirable to adapt it to the determination of manganese.

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Experimental

The common methods for the final determination of manganese in minute quantities, as it is found in plant material, involve the oxidation of bivalent manganese to heptavalent manganese by some oxidizing agent stronger than the permanganic ion, such as periodic acid, with a subsequent colorimetric comparison of the permanganic ion. Chlorides interfere with this reaction and are commonly present in rather high concentration, especially in mixed feeds, but the use of perchloric acid readily eliminates this difficulty. The products of decomposition of perchloric acid (*8*) are chlorine and oxygen. Chlorides are volatilized either as hydrogen chloride or as chlorine after oxidation. Remaining small traces must be eliminated by boiling in the presence of sodium periodate before the color of permanganic acid will develop.

Richards (*7*) has shown that when a small amount of manganese is to be determined there should be from 5 to 6 per cent of sulfuric acid in the solution previous to adding periodate to develop permanganic acid color. When this same percentage of perchloric acid was present, however, at the time of development of color there was considerable fading and sometimes precipitation of manganese dioxide. To determine the optimum quantity of perchloric acid required, equal amounts of a standard manganese solution were added to 0, 1, 5, 10, and 15 per cent perchloric acid solutions. On adding sodium periodate and heating there was a rapid precipitation of manganese dioxide in the 0 and 1 per cent solutions, some fading in the 5 per cent, but none in the 10 or 15 per cent solutions.

Table I. Recovery of Manganese Following Digestion with Perchloric Acid

Weight of Sample[a]	Standard Manganese Solution	Digestion Period	Manganese per 100 Ml. of Solution	Recovery
Grams	*Ml.*	*Min.*	*Mg.*	%
4.000	None	15	0.225	
			0.218	
			0.225	
			Av. 0.224	
4.000	25.00	15	0.410	
			0.420	
			0.460	
			0.455	
			Av. 0.4362	90.2
4.000	25.00	45	0.455	
			0.455	
			0.455	
			Av. 0.4550	98.3
4.000	25.00	80	0.455	
			0.450	
			Av. 0.4525	97.2
None	25.00	15	0.235	
			0.235	
			Av. 0.2350	100.0
None	25.00	None	0.235	
			0.235	
			Av. 0.2350	

[a] Sample used for this study was a mixed poultry feed.

Procedure

Weigh a suitable quantity of plant or feed material, usually 3 to 4 grams, and transfer it to a 500-ml. Kjeldahl flask. Add 40 ml. of concentrated nitric acid and boil gently over a gas flame. Avoid heating the flask above the surface of the liquid by placing it on an asbestos board with a hole approximately 4 cm. in diameter. When the sample is nearly dry (avoid burning the sample) remove the flask from the heat, add 13 ml. of 60 per cent perchloric acid, and again boil gently. When the remaining nitric acid and water are driven off there is a vigorous evolution of gas, the solution boils rapidly, and white fumes appear. Just previous to the appearance of white fumes turn down the gas flame as low as possible, so that it is barely touching the flask, and continue the digestion for 10 to 15 minutes; boiling need not be rapid. Remove from the flame, allow to cool for 5 to 10 minutes, add a few milliliters of water, and filter on an asbestos pad (the asbestos must be digested with potassium permanganate and acid-washed) or on a sintered-glass funnel into a 150-ml. beaker. Wash with about 50 ml. of water, add 0.3 gram of sodium periodate, boil for about 2 to 5 minutes with stirring to avoid bumping, and place on a steam bath for about 0.5 hour. Transfer to a 100-ml. volumetric flask, make up to volume with boiled distilled water, and compare in a colorimeter against a standard solution of potassium permanganate of approximately equal concentration. A blue filter aids in this color comparison.

Discussion

Kahane (*6*) showed that a wet combustion using a nitric-sulfuric-perchloric acid digestion of organic matter gave a loss of chromium and manganese by entrainment in the vapors from the digest. In order to determine whether such a loss was significant in studying animal feeds an experiment was set up, the outline and results of which are shown in Table I. The colorimetric readings in this experiment were made with a photoelectric colorimeter that had been standardized with standard potassium permanganate solutions. There was good recovery of added manganese, ranging from 90 to 98 per cent when organic matter was present, and 100 per cent recovery when no organic matter was present.

Table II gives the results of another recovery study and a comparison of the perchloric acid method and the method of the Association of Official Agricultural Chemists (*1*) The recovery of added manganese was very satisfactory. The average of group 1 0.158 mg. per 4 grams, plus the average of group 3, 0.110 mg. per 4 grams, equals 0.268, which is essentially the same as the average of group 2, 0.271. A comparison of groups 3 and 4 shows again that in the absence of organic matter there was no loss of manganese during digestion. A comparison of groups 1 and 5 shows that the A. O. A. C. method gives materially lower values than the perchloric acid method. This is in line with the report of Bolin (*2*) and Davidson (*3*) that in the case of dry-ashing procedure a sodium carbonate fusion or silica volatilization is necessary to recover all the manganese. Smith (*9*) stated that the A. O. A. C. method included all acid-soluble forms of manganese in the ash of feeds and that it seemed unlikely that compounds excluded by this treatment are of nutritional value. However, he presented no data and the question needs investigation. It would also be desirable to have data comparing the recovery of manganese using the perchloric acid digestion and a sodium carbonate fusion and a silica volatilization.

Serious explosions have been known to occur from the use of perchloric acid in the oxidation of organic matter, but the author has experienced no accidents in many hundred perchloric acid digestions However, great care should be exercised in varying the procedure materially, especially the use of larger samples without greater nitric acid oxidation and possibly more perchloric acid. If it is desirable to use much larger samples than indicated, it probably would be better to use a nitric-sulfuric-perchloric oxidation, but this is more time-consuming and needs closer attention throughout the digestion period.

During the digestion with nitric acid alone the solution should be boiled slowly so that it requires 30 to 40 minutes to evaporate the 40 ml. of nitric acid. It is possible otherwise to evaporate the nitric acid and not oxidize the material

Table II. Compaison of Perchloric Acid Method with A. O. A. C. Method for Manganese

Group No.	Description	Manganese in 4-Gram Sample
		Mg.
1	4 grams of standard feed sample[a] (digested with HNO_3 followed by $HClO_4$)	0.152
		0.165
		0.160
		0.158
		0.155
		0.158
		Av. 0.158
2	4 grams of standard feed sample + 10 ml. of $MnSO_4$ solution containing approximately 0.1 mg. of manganese (digested as in group 1)	0.255
		0.245
		0.255
		0.280
		0.275
		0.300
		0.288
		Av. 0.271
3	10 ml. of $MnSO_4$ solution + 10 ml. of $HClO_4$ (digested as in group 1)	0.110
		0.105
		0.105
		0.104
		0.118
		0.118
		Av. 0.110
4	10 ml. of $MnSO_4$ solution (no digestion with $HClO_4$)	0.115
		0.118
		0.116
		Av. 0.116
5	4 grams of standard feed sample. Manganese determined by A. O. A. C. method	0.140
		0.138
		0.140
		0.145
		0.138
		0.125
		Av. 0.137

[a] Mixed poultry feed.

sufficiently. If copious nitrogen peroxide fumes are still visible in the flask when the sample is nearly to dryness, as will often be the case when fish meals or oily substances are present, another 15 ml. of nitric acid should be added and the slow digestion continued. Care should be taken to avoid burning or charring the sample. All the perchloric acid should be added at one time. Precautions of Kahane (*5*) are: (1) Oxidize as much as possible with nitric acid before adding the perchloric acid because it is the easily oxidized material that reacts vigorously with perchloric acid, and (2) always add an excess of perchloric acid and never boil the solutions too rapidly.

Acknowledgment

The author wishes to thank James Maddox, student, for his technical assistance.

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Boron Determination in Volatile Organic Compounds

Using the Parr Oxygen Bomb

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A REVIEW of the literature regarding the methods for the determination of boron in organic compounds reveals the fact that some of the methods are either lacking in accuracy or are long and cumbersome. In foods and fertilizers boron is usually present as boric acid or borax, which are less volatile than the aliphatic boron compounds, and the organic matter may be destroyed by ashing in the presence of an alkaline substance. This method (*2*) obviously cannot be applied to organic compounds which volatilize before decomposing or oxidizing.

Several attempts have been made to analyze for boron when fluorine is present. Bowlus and Nieuwland (*3*) analyzed boron fluoride for boron with an average error of about 6 per cent, using the Carius method of decomposition. Pflaum and Wenzke (*5*) used the Parr fusion bomb with an oxidizing mixture of sodium peroxide, sugar, and potassium chlorate. The high concentration of alkali caused difficulties in the determination of fluorine, which were overcome by adding ammonium chloride. Then in preparation for the boron determination, more strong sodium hydroxide was used to remove the ammonium ion. Snider, Kuck, and Johnson (*6*) used the Parr fusion bomb for the determination of boron in boronic acids. They obtained good results, but the fusion bomb has the limitations of a maximum sample of about 0.3 gram and the introduction of larger amounts of reagents; these limitations are not necessary in decomposing aliphatic boron compounds by the oxygen bomb method described below. The digestion with hydrogen peroxide and the subsequent evaporation and fusion with sodium hydroxide are time-consuming, and still involve the use of large amounts of reagents.

Improved Oxygen Bomb Method

An accurately weighed sample of approximately 1 gram is used in the Parr oxygen bomb according to A. S. T. M. Method D-129–39 (*1*), with the addition of about 1 gram of sodium carbonate to the water in the bomb. Volatile substances should be weighed in a gelatin capsule. After the oxidation, the alkaline solution containing the boron in the form of sodium borate is evaporated to about 25-ml. volume, transferred to a 500-ml. Erlenmeyer flask, and then acidified with 3 *N* hydrochloric acid using methyl red indicator and 3- to 5-ml. excess of acid. The solution is boiled for about 20 minutes under a reflux condenser to liberate carbon dioxide, cooled, and brought to neutrality with carbonate-free sodium hydroxide. Then 0.1 *N* hydrochloric acid is added until the sample is just pink to methyl red, and the solution is finally titrated with 0.1 *N* sodium hydroxide in the presence of mannitol, using phenolphthalein as an indicator. Results are calculated thus:

$$\% \text{ boron} = \frac{\text{ml. of 0.1 } N \text{ NaOH required} \times 0.001082 \times 100}{\text{weight of sample}}$$

According to Hillebrand and Lundell (*4*) the boric oxide titer of sodium hydroxide is not accurate when calculated directly from the sodium hydroxide content; hence the 0.1 *N* sodium hydroxide was standardized using pure dry boric oxide. A blank test was run on the reagents used, determining the differences between the methyl red and phenolphthalein

Table I. Results of Analysis

Weight of Sample	Titration	Blank	Net Titration	Boron
Grams	*Ml.*	*Ml.*	*Ml.*	*%*
Amyl Borate (B. P. 122–123° C., 3 Mm. Hg Pressure). NaOH (0.1001 *N*), 1 Ml. = 0.001083 Gram of Boron				
1.0270	38.04	1.09	36.95	3.90
1.0861	40.69	1.09	39.60	3.91
1.1719	43.49	1.09	42.40	3.95
1.0280	38.29	1.09	37.20	3.92
			Average	3.92
			Theoretical	3.97
Amyl Borate (Eastman Practical). NaOH (0.1020 *N*), M1 l. = 0.001103 Gram of Boron				
1.0060	36.90	1.09	35.81	3.93
1.0221	37.50	0.80	36.70	3.96
1.0230	37.40	0.80	36.60	3.95
			Average	3.95
			Theoretical	3.97
n-Butyl Borate (Eastman White Label). NaOH (0.0994 *N*), 1 Ml. = 0.001076 Gram of Boron				
1.2103	53.35	1.05	52.30	4.65
1.0321	45.90	1.05	44.85	4.68
1.0214	45.38	1.05	44.33	4.67
1.0815	48.20	1.05	47.15	4.69
			Average	4.67
			Theoretical	4.70

end points. The accuracy obtained was about one part in 100 of the percentage of boron found. Using this procedure the analyses presented in Table I were performed.

Summary

The Parr oxygen bomb is satisfactory for the combustion of organic boron compounds in preparation for the determination of boron. It provides a means for decomposing organic boron compounds without the use of large amounts of reagents, and gives equal or greater accuracy than some methods heretofore proposed.

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Oxidation of Lubricating Oils

Apparatus and Analytical Methods

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An apparatus and procedure are described which permit determination of the rate of lubricating oil oxidation. Studies are usually made over the range 130° to 180° C. using oxygen in a circulatory system. Methods have been developed for the determination of substantially all the volatile and nonvolatile end products of the reaction.

Ninety to 100 per cent of the oxygen absorbed by various oils has been accounted for in the products determined. The chief product is water, which accounts for 40 to 60 per cent of the reacting oxygen. Soluble saponifiable materials represent another considerable portion, while carbon dioxide, carbon monoxide, volatile acids, fixed acids, and precipitable products account for the remainder.

The analysis of insoluble materials produced by laboratory and engine oxidation indicates these to be relatively highly oxygenated materials (14 to 24 per cent oxygen) produced by polymerization or condensation of the oxidized oil. Molecular weights and elementary analyses suggest certain empirical formulas for the insoluble bodies.

The oxidation of several commercial light oils shows that rates of oxidation vary widely and that induction-type oxidation curves may be common. In general, these results show the widely different oxidation characteristics existing in oils, and that much of the oxidation mechanism remains to be understood before corrective measures are generally applicable.

MODERN developments in the refining of lubricating oils for internal combustion engines have produced many changes in the methods of their preparation. Within the past few years the stability of such oils toward deterioration in use with the formation of harmful products has become a problem of considerable importance. This may be attributed to several circumstances. Variations in engine design intended for more economical operation have reduced oil consumption, and consequently the amount of make-up oil which must be added between drain periods. At the same time manufacturers have tended to recommend longer mileages between drains, which also has prolonged the period of exposure of the oil to deterioration.

During the past ten years in automobile engines the horsepower per cubic inch of displacement has increased 30 per cent, the average compression ratio 26 per cent, the average brake mean effective pressure at maximum horsepower 13 per cent, and the brake horsepower 25 per cent. In general, such increases lead to increased engine block and crank case temperatures. Along with these more severe conditions have come the introduction of certain bearing alloys more susceptible to corrosion than the conventional babbitt bearings, and the use of decreased clearances, which necessitate that the lubricant shall not produce deposits that will cause the pistons or valves to stick within their operating passages.

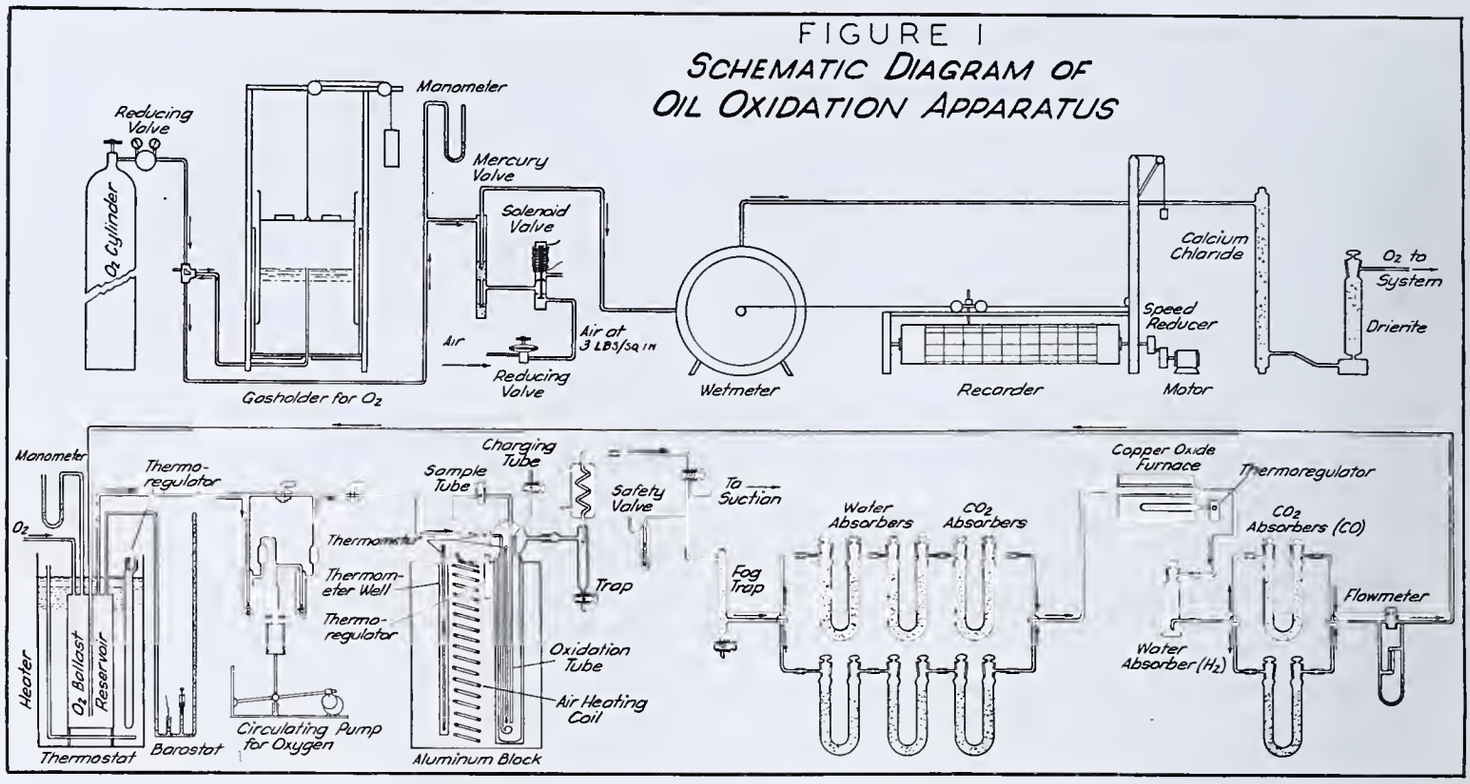
It has been shown many times (*5*, *12*) that the major effects of oil deterioration are those due to oxidation of the lubricant. Most studies of oil deterioration have involved one or another of numerous arbitrary and relatively simple oxidation tests. In some of these [Michie (*2*), Sligh (*16*), Indiana (*14*), etc.] a standard quantity of oil is subjected to air or oxygen (sometimes under pressure) at a set temperature, with or without catalysts, and such values as acid number, sludge, tar formation, oxygen absorption, viscosity change, etc., are measured as an index of the degree of deterioration. Many attempts have been made to correlate such laboratory tests with engine tests on various oils, but failure to do so is signally evident.

A logical approach to an understanding of the mechanism of oil failure in service is to study oils while oxidizing under controlled conditions, and to determine the extent and kinetics of the oxidation reaction and, in so far as possible, the amount and nature of all the products. Such studies on insulating oils have been made by Balsbaugh (*4*). However, relatively few workers have conducted extensive oxygen absorption tests, and it is particularly unfortunate that some who have made such tests have failed to describe adequately the oils with which they worked. In other cases no attempt was made to indicate the nature of the materials produced by such oxidation.

One of the aims of this work was to secure such an understanding of the oxidation reaction that it would be possible to write equations which would indicate the quantities of all the oxygenated end products. This does not imply that the structure and chemical nature of all these materials need to be known, but only that it should be possible to classify them, to determine quantitatively the classes present, and to calculate the complete oxygen balance from a knowledge of the oxygen content of each of these classes.

The apparatus used in this investigation is an elaboration of a simpler type used by Dornte (*8*). Means were provided for circulating oxygen, or oxygen-containing gas mixtures, through a quantity of oil maintained at a constant tempera-

FIGURE 1
SCHEMATIC DIAGRAM OF OIL OXIDATION APPARATUS



ture, for continuously removing volatile oxidation products from the circulating gas as formed, for supplying oxygen to make up for that consumed by the oil, and for making a continuous record of the amount of oxygen so supplied. The device has certain features of design which make it very flexible and adaptable to numerous variations in the technique of oxidation. In addition, the application of automatic control devices permits it to be used with a minimum of supervision for most purposes. In general, while operating, little or no attention is required. Consequently, the use of an overnight period greatly facilitates the productivity of the apparatus.

Apparatus for Oxygen Absorption Tests

A schematic diagram of the apparatus is shown in Figure 1. The two sections shown, respectively, in the upper and lower halves of the diagram, are actually situated end to end, with the lower section continuing from the right-hand end of the upper half. The apparatus shown in the lower portion comprises the closed system in which oxygen is circulated through the oil and from which gaseous reaction products are removed as formed. The upper half shows the means whereby pure dry oxygen, to make up for that consumed, is supplied to the closed system automatically maintained at a constant pressure, together with the device for graphically recording the amount of oxygen so supplied.

Oxygen Supply and Recording System. Commercial cylinder oxygen is delivered when required at reduced pressure to an inverted bucket gasholder of about 25 liters' capacity, where it is kept over water at about 1.25 cm. (0.5 inch) of mercury above atmospheric pressure. This pressure is provided by weights on top of the bucket. Oxygen is withdrawn from the gasholder and supplied to the closed system when required by the action of a mercury barostat and an air-operated, solenoid-controlled mercury valve.

The original design of the apparatus contemplated using a solenoid valve directly to admit oxygen to the system. Several valves were tried and found to leak slightly, including a spring-seated type. To overcome this trouble a positive-seal mercury valve was developed as shown in Figure 2. This consisted of two chambers: the lower a mercury reservoir, and the upper the valve chamber. The valve was closed (the normal position) to passage of oxygen by applying line air pressure reduced to 2109 kg. per sq. meter (3 pounds per square inch) to the mercury reservoir. This forced mercury into the upper chamber above the level of the oxygen outlet; the mercury thus prevents passage of oxygen through the valve. A three-way solenoid valve was applied to open the mercury valve, by closing the passage between the mercury reservoir and the air line and opening the reservoir to the atmosphere. When this took place the mercury flowed from the upper chamber into the reservoir, and permitted oxygen to pass through to the apparatus. Using relatively large ducts and short distances of travel for the mercury, the operating cycle is complete in an instant.

The recording mechanism consists of a wet-test gas meter through which the oxygen passes, and a drum which is rotated at a rate of one revolution in 60 hours. A set of tracks and a small carriage carrying a pencil are mounted above the recorder drum. The passage of gas through the wet meter winds a cord on a shaft mounted on the dial of the meter in place of the usual indicating hand. As the cord is wound, it draws the carriage axially along the drum, and the pencil produces a line of oxygen consumption against time on a large sheet of millimeter graph paper clamped to the drum surface. The cord shaft and drum are so dimensioned that 1 cm. in the direction of rotation corresponds to 1 hour's time, and 1 cm. axially corresponds to the consumption of 500 cc. of oxygen. Rotation of the drum is produced by a small constant-speed 2400 r. p. m. motor driving two geared speed reducers (2400/1 and 3600/1) in series. The useful length of the drum is 50 cm., corresponding to the consumption of 25 liters of oxygen.

The oxygen after passage through the gas meter flows into a pair of drying tubes containing calcium chloride and anhydrous calcium sulfate, respectively, before being supplied to the closed system.

Closed System for Circulating Oxygen Through Oil. This consists of a ballast reservoir for oxygen, a circulating pump, the oxidation tube containing the oil under investigation, and a series of absorbers for removing oxidation products from the circulating gas.

The ballast reservoir reduces pressure fluctuation due to the pump stroke, and has a volume of 2600 cc. It is immersed in a water thermostat held at 40° C. Oxygen is withdrawn from the reservoir for circulation by a packless pump equipped with mercury check valves. The "cylinder" of the pump is a flexible copper bellows 5 cm. (2 inches) in diameter and about 7.5 cm. (3 inches) long which is alternately compressed and extended by means of a motor-driven cam-and-lever arrangement. The maximum stroke of the bellows is 1.25 cm. (0.5 inch), and this can be decreased to control the pumping rate by means of an adjustable stop on the lever. Usually the pump circulates about 15 liters of oxygen per hour.

The oil sample undergoing oxidation is contained in a glass tube, situated in a well in a cast aluminum block thermostat. The size of the sample may be varied, but is normally 200–250 grams. Since the first runs were made using 250-gram samples, all data have been calculated and reported in terms of the 250-gram charge, regardless of the actual sample size.

A sketch of the glass oxidation tube is shown in Figure 3. It consists of two parts: the tube proper, and the cap shown in two sections at right angles in the figure. The two are joined with a silver-clad ground-glass joint. The cap is supplied with an oxy-

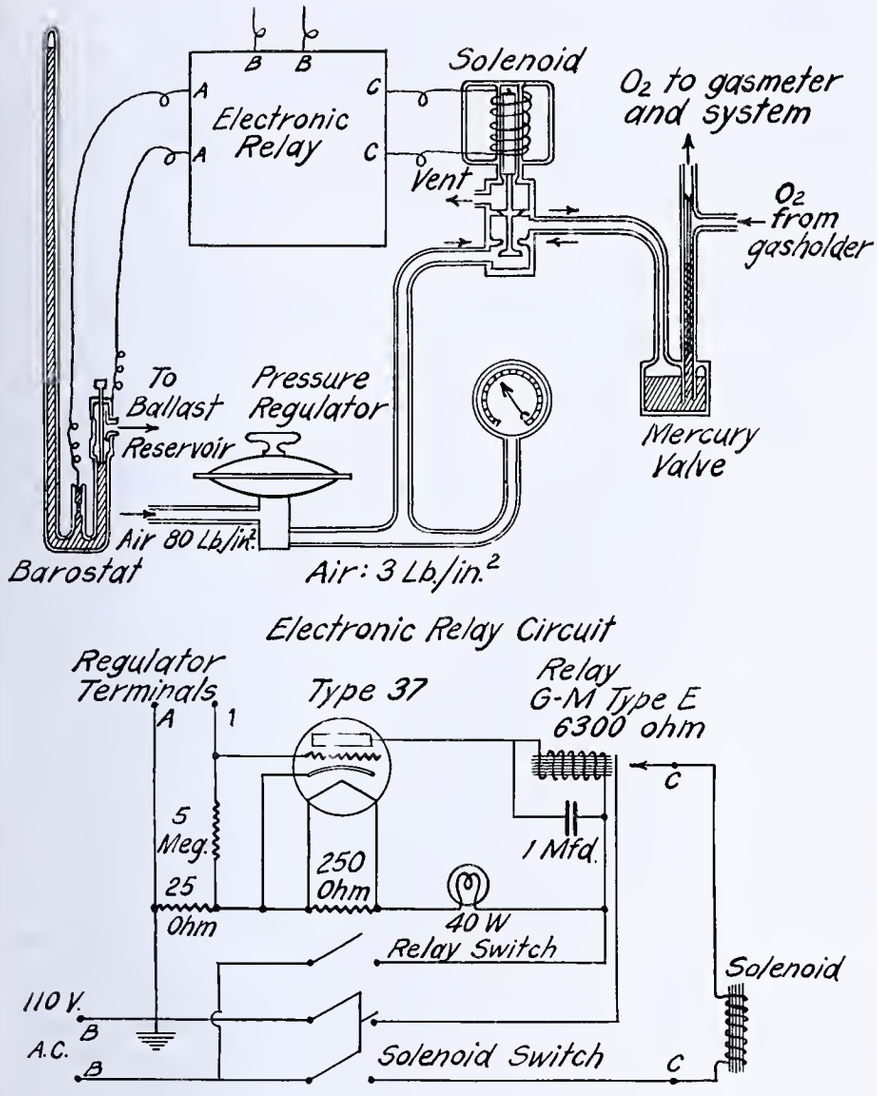


FIGURE 2. MERCURY VALVE AND OPERATING SYSTEM

gen inlet, an oxygen outlet, a tube for the withdrawal of a sample while the run is in progress, and a tube for admitting the oil charge. The oxygen inlet tube terminates in a bulb supplied with perforations positioned horizontally about its circumference, which break up the incoming gas stream into small bubbles. A sintered-glass disk was first used for this purpose but was replaced because it soon became clogged with sludge particles. The end of the sampling tube terminates just above this bulb. On the side of the sampling tube and about 12 cm. from the bottom a small hook is placed, from which a microscope slide may be hung to determine lacquering tendency.

The charging tube ends just below the ground-glass joint in the cap. Charging of the oil is a degassing operation as well. The oxidation tube is closed off from the remainder of the apparatus and evacuated by means of a water aspirator, while the block is brought to the operating temperature or slightly higher. The charge of oil is then dropped slowly into this hot partially evacuated space over a period of about an hour while maintaining the suction.

ABSORPTION SYSTEM. The gas leaving the oxidation tube containing oxygen plus volatile oxidation products passes first to a water-cooled condenser and trap. The trap usually collects a small quantity of oil during a run (0.3 to 3.0 cc., depending upon the volatility of the charge) but ordinarily no aqueous condensate is obtained unless the oxygen absorption rate exceeds about 400 cc. per hour. Because of the temperature of the water-cooled trap, a partial pressure of water vapor in the gas in excess of about 18 mm. is needed before water condenses out of the gas. To create this partial pressure the oxygen absorption rate would have to be 400 cc. per hour or greater when the gas circulation rate is about 15 liters per hour and about half of the oxygen absorbed reacts to form water. The trap product, particularly the aqueous layer, normally contains a moderate to large quantity of volatile acids, whose analysis is reported below. Volatile organic acids may constitute up to 30 per cent of the aqueous condensate.

WATER. The absorbers for removing the gaseous oxidation products, such as water, carbon dioxide, and carbon monoxide, are provided in duplicate circuits with appropriate stopcocks to permit either set to be used during the run. Thus it is possible to follow the rate of production of each of these materials, since one set of absorbers may be taken off and weighed at regular intervals, while the other set is in operation. Significant variations occur in the rate of production of these materials, even for oils whose rate of oxidation is substantially constant. Thus, in the oxidation of a distillate oil at 140° C., water is the first volatile product to be detected, then successively carbon dioxide and carbon monoxide. Within a few hours in such an oxidation the proportions of these products rise to their normal percentages, which thereafter show little change.

The selection of a satisfactory water absorbent was difficult. Materials tried were phosphorus pentoxide on pumice, barium perchlorate, magnesium perchlorate, calcium chloride, and anhydrous calcium sulfate (Drierite). Calcium sulfate (8-mesh) was the final choice because it permitted the simultaneous determination of volatile acids, lacked the explosion hazard of the perchlorates, and was relatively cheap and convenient to handle. Because of the low capacity and apparent slower absorption of this material, 20-cm. (8-inch) Schwartz U-tubes are used in series for water removal. The first tube usually removes at least 95 per cent of the water.

The determination of volatile acids on the water absorbent was done simply by grinding the absorbent with water, filtering and carefully washing, and titrating the filtrate with standard alkali. This determination then became a regular part of the oxidation procedure, and a correction was applied to the apparent amount of water recovered to allow for volatile acids weighed as water.

CARBON DIOXIDE. Removal of water from the circulating gas was followed by absorption of carbon dioxide. An absorber of the same design was used for this purpose, one leg of which was packed with Ascarite (potassium hydroxide on asbestos) and the other with the water absorbent. The gas when leaving the carbon dioxide absorber passed through the calcium sulfate–filled leg to ensure it possessing the same concentration of water vapor as when it entered from the water absorber.

Ascarite is satisfactory for the absorption of carbon dioxide, and no other material was tried. Its capacity is at least several grams of carbon dioxide per tube and when tested by following it

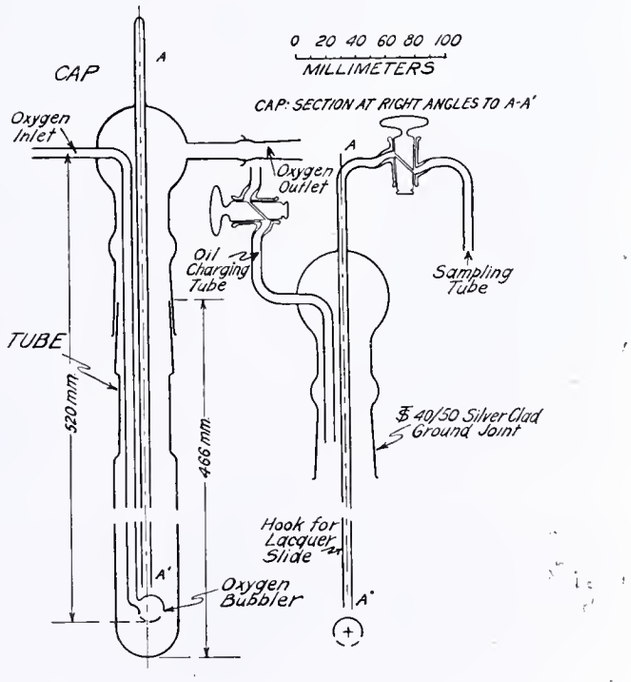


FIGURE 3. OXIDATION TUBE AND CAP

Table I. Analyses of Circulating Gas from Ballast Reservoir after Oxidation

Run No.	9	10	11	12	20
Temperature, ° C.	165	165	165	165	170
Oxygen absorbed in 50 hours by 250 grams of oil, millimoles	261	263	222	274	344
Gas analysis					
O_2, %	75.5	74.5	78.3	74.5	91.6
Illuminants[a], %	0.0	0.0	0.0	0.0	0.0
CO, %	6.1	6.7	6.0	8.1	0.5[b]
H_2, %	0.0	2.5	1.5	2.2	0.3
Hydrocarbons (as ethane), %	1.4	0.2	0.3	0.3	0.4
Nitrogen (by difference), %	12.6	14.0	12.7	14.3	7.0

[a] Absorbed in fuming sulfuric acid.
[b] After installation of copper oxide furnace.

with an additional absorber, the single tube appeared to remove carbon dioxide with substantial completeness.

Carbon Monoxide. It was not at first planned to determine or remove carbon monoxide from the circulating gas, as other investigators (Müller, Dornte, Clarke) have indicated that this gas is either absent or present in relatively small quantities. It was found by analyses of a terminal sample of the circulating gas that a significant amount of carbon monoxide always accumulated in the system (Table I) and that small quantities of hydrogen and paraffin hydrocarbons were present. The purity of the oxygen in the ballast reservoir at the start was usually about 95 per cent. Additional nitrogen was probably introduced by interchange with air when absorbers were changed during progress of the run. A small percentage also entered with the inlet oxygen. Since it was desired to maintain the oxygen partial pressure at as high and as constant a value as possible, some means of removing carbon monoxide as formed was required.

This was done by installing a copper oxide furnace in the absorption circuit following the water and carbon dioxide absorbers. The furnace consisted of a 12.5-cm. (5-inch) piece of 0.938-cm. (0.375-inch) iron pipe packed with wire-form copper oxide and situated in an aluminum block held at 330° to 350° C. It was preceded by an absorption tube containing activated charcoal for removal of hydrocarbons, and was followed by an additional pair of water and carbon dioxide absorbers.

The removal of hydrogen and carbon monoxide by catalytic combustion was reasonably effective, as seen in the data for run 20, Table I. Data in this table refer to the oxidation of a distillate oil of 183 Saybolt seconds viscosity at 100° F., and a viscosity index of 100. The furnace is operated at a temperature high enough to allow for slow burning of paraffins such as propane, etc., and it is possible that a portion of both the carbon dioxide and water recovered following the copper oxide furnace should be attributed to the combustion of hydrocarbons. There is, however, insufficient water produced to account for more than a part of the carbon dioxide from this source. There is normally only a small absorption of material on the charcoal, but the quantity is variable and may be due to water, oxygenated compounds, entrained oil droplets, etc.

Volatile Acids. These are recovered from several sources. They result principally from washing the water absorbent, and from any oily or aqueous condensate collected in the trap. They occur in the trap water layer only when the oxidation is so rapid as to evolve a gas containing more than enough water vapor to saturate itself at the condenser exit temperature. Volatile acids appear with the water and may constitute 20 to 30 per cent of the aqueous layer. Small quantities may be found in any oily trap condensate, but usually not in significant quantities in the absence of an aqueous condensate.

Recovery of volatile acids from the Drierite (calcium sulfate) is aided by the water insolubility of this substance. The absorbent is ground with enough water to form a heavy paste and this is filtered quickly or kept agitated so that the plaster of Paris formed does not have an opportunity to set. The mass is suction-filtered using a Büchner funnel, and is thoroughly washed with water. The filtrate and washings are then titrated with 0.1 *N* sodium hydroxide. All the acid is retained by the first of the two water absorbers and the absorbent in the second tube is ordinarily not washed or titrated. To determine the acidity of the trap product comprising the oily and aqueous layers, these are titrated together if the quantity is small—for example, less than 1 cc. Some of the oil may disappear when this is done. This is evidently an indication that water-insoluble acids of higher molecular weight are present. When a larger quantity of aqueous layer is present, an aliquot is titrated and then the oil layer is titrated separately.

The sum of these acidity figures is used in computing the fraction of the oxygen absorbed which appears in the product as volatile acids. It is possible that some volatile acid remains in the oxidized oil, but considering the high rate of oxygen circulation through the oil and the high oxidation temperatures used, this quantity must be small.

The determination of volatile acids is also necessary in order to correct the apparent weight of water produced. To do this, it was necessary to make an estimate of the average molecular weight of the acids. The acids were first isolated by evaporating the neutralized washings, or neutralized trap product, to a small volume, then adding phosphoric acid to liberate the volatile acids from their salts, and distilling the acids out of the mixture.

The composition of the volatile acids was estimated by means of the DuClaux procedure (*9, 17*). This method is distinctly empirical; its value is in its simplicity and the small quantity of material required. The composition of an unknown mixture of more than two component acids can be estimated by attempting to match the curve for the titration of successive increments of distillate with known mixtures of acids which may be assumed to be present. A mixture of formic, acetic, propionic, and butyric acids in molecular proportions of 3 to 5 to 0.7 to 1 (average molecular weight 60) gave a curve which followed well that for the unknown acid mixture. Therefore an average molecular weight of 60 was assumed for volatile acids. There appears to be little variation in the relative amounts of the different volatile acids obtained in the oxidation of various oils. It is probable that the proportion of formic acid is somewhat higher than indicated above, since direct determination of this acid by its reducing action on mercuric chloride, causing precipitation of mercurous chloride (*3, 15*), indicated that the amount of formic acid ranged between 40 and 50 per cent of the total volatile acids.

Volatile Carbonyl Compounds. It was to be anticipated that since volatile organic acids are produced in oil oxidation, other volatile oxygen-containing products would also be formed. Several of the aqueous trap products were tested for the presence of aldehydes with Schiff's reagent (rosaniline hydrochloride solution decolorized with sulfur dioxide) and positive tests were obtained. The amount of aldehydes and ketones present was determined on an aliquot of the trap product by means of the Bryant and Smith hydroxylamine hydrochloride procedure (*6*). These data were obtained for only a few of the runs and a summary of the values secured is given in Table II along with the quantity of volatile acids present in the same materials. The oils shown here are narrow, relatively homogeneous fractions produced by distillation and extraction of a light distillate oil.

The trap products from the first four runs listed in Table II are essentially similar in composition and were produced by the oxidation of light, highly paraffinic oils. Carbonyl oxygen thus determined accounts for only 0.6 to 1.0 per cent of the total oxygen absorbed. No measurement of the amount of carbonyl compounds retained by the water absorbers was made, but this is probably considerably less than that found in the trap.

The trap product from run 62 was much smaller in amount and contained a smaller proportion of volatile acids and a much smaller proportion of carbonyl oxygen. This product was obtained from the oxidation of a heavy distillation residue high in aromatic ring content. Since volatile carbonyl oxygen

was demonstrated to be small in amount, no correction was made for it in the estimation of the water formed.

Calculation of Corrected Oxygen Absorption. The apparent oxygen absorption indicated by the recorder chart is subject to several corrections in determining the actual amount of oxygen which has reacted with the oil.

The oxygen content of the circulating gas is determined initially and at the completion of each run. It is then assumed that the decrease in the oxygen content represents oxygen consumed by the oil but not measured by the recorder—i. e., oxygen which is consumed and replaced by some other gas. This would be correct if the replacing gas were some product of the oxidation, as paraffinic or olefinic hydrocarbons, etc. It is almost wholly true (the right value would be 80 per cent of the difference) if the replacing gas is air entering from a leak. It is not true at all if the drop in oxygen content is due to nitrogen present as an impurity in the oxygen which passes through the recorder. However, with oxygen which was 99.5 per cent pure, a total absorption of 20 liters would be required to cause a 3 per cent decrease in the oxygen content of the 3300 cc. of circulating gas. The correction is made as first assumed and computed in terms of cubic centimeters of oxygen at 0° C. and 760 mm. of mercury.

For oxygen utilized in the combustion of carbon monoxide and hydrogen in the copper oxide furnace, a correction is made on the basis of the following equations:

$$2CO + O_2 = 2CO_2$$
$$2H_2 + O_2 = 2H_2O$$

Two millimoles (0.088 gram) of carbon dioxide, or 2 millimoles (0.036 gram) of water, recovered after the copper oxide furnace are each equivalent to 1 millimole (22.4 cc. at standard conditions) of oxygen which was consumed other than by the oil. This correction consequently is subtracted from the apparent oxygen consumption and more or less completely compensates for the first correction.

The oxygen passes through the wet meter at varying temperatures, so that its water vapor content depends upon the ambient temperature. It is assumed that it is saturated with water at the temperature of the wet meter. During the course of a run the wet-meter temperature is recorded at four to ten well-spaced intervals, and the corresponding water vapor partial pressures are read from aqueous tension tables. Both temperatures and partial pressures are then averaged and these average values used to determine the volume of oxygen absorbed when calculated to standard conditions of temperature and pressure.

Analysis of the Oxidized Oil. Treatment of the oxidation product was directed in part to determining the distribution of the oxygen absorbed among the products of the reaction, in order to afford some understanding of the oxidation mechanism. A consideration of equal importance was the measurement of the significant characteristics of the oxidized oil—namely, "dirtiness" such as insolubles and lacquer, acidity, and viscosity change.

A clarified oil sample was produced from the oxidation product simply by filtering off products which were insoluble in the oxidized oil at room temperature by the use of Jena sintered-glass filters of fine porosity (G-4). Some of the oil-soluble oxidized materials were then removed by precipitation with isopentane at about 15° C. to produce an isopentane-treated oil sample. This separation of oil-soluble and oil-insoluble precipitables was based on a procedure suggested by Levin (*11*). The total quantity of precipitables was determined on a well-shaken sample of the oxidation products. The oil-soluble, isopentane-insolubles were determined separately on the clarified oil sample from which the oil-insolubles had been filtered as mentioned previously. By subtracting the amount of oil-solubles from the amount of total insolubles, a value for the quantity of oil-insolubles was obtained.

The procedure for the determination of precipitables consists in diluting an approximately 10-gram oil sample with 10 volumes of isopentane and allowing to stand overnight in a bath of cold water in order to minimize loss of solvent. The mixture is filtered through an asbestos mat on a Gooch crucible and the precipitate is washed with several additional volumes of isopentane. The crucibles are dried in an oven at 150° C. to constant weight (usually overnight). The isopentane-treated oil is recovered from the determination of both the total insolubles and the oil-solubles by combining the filtrates and stripping off the isopentane.

Determination of Lacquer-Forming Tendency. One of the problems prompting a study of the oxidation characteristics of lubricating oils was due to the deposition of a red-brown varnish- or lacquerlike film on piston or other engine surfaces. This was presumed to be responsible, at least in part, for the sticking of piston rings, valves, and even of the pistons in the block. This type of deposit had long been recognized by investigators in the oil and automotive fields, but was not as critical a problem with lower operating temperatures and greater clearances. In the early stages of these laboratory oxidation studies, the deposition of a superficially similar film on the wall of the glass oxidation tube was observed. This material is also varnishlike and it has been assumed, lacking evidence to the contrary, that its formation is an oxidation phenomenon related to engine varnishing. It was found possible to remove this lacquer from the glass surface readily and almost quantitatively by first carefully removing any oil by washing with naphtha or isopentane, drying, and then treating with water, upon which the lacquer deposit came off in flakes. The lacquer was filtered from the water suspension, dried, and weighed. The quantity of material secured in this manner was small, amounting to only 0.1 to 0.3 per cent by weight of oil charged to the apparatus.

It was suspected that the formation of this deposit was a surface phenomenon, and a simple lacquer-forming tendency test was devised. This consisted in suspending a standard 3 × 1 inch (75 × 25 mm.) microscope slide in the oil undergoing oxidation, and weighing the deposit which the slide acquired. A 0.625-cm. (0.25-inch) hole was drilled in the glass plate about 1.25 cm. (0.5 inch) from one end to make it easier to suspend. Since the amount of the deposit was always small, it was necessary to clean and dry the glass very carefully and to weigh with four-place accuracy (±0.0002 gram). All oil was removed from the lacquered slide by washing with naphtha and any adherent insoluble particles were gently rubbed off. The glass was then oven-dried at 105° C. and kept in a desiccator until reweighed. Results are

Table II. Composition of Volatile Condensable Material

(Basis: 250 grams of oil oxidized at 140° C. for 50 hours with oxygen at approximately 700 mm. partial pressure)

Run No.	52	54	55	59	62
Properties of oil oxidized					
Viscosity, centistokes at 100° F.	15.54	21.39	38.96	39.13	312.5
Kinematic viscosity index	134	137	125	126	45
Average molecular weight	326	367	443	446	494
Oxygen absorbed by oil, millimoles	1622	1200	1982	1103	729
Composition of trap product					
Amount recovered, grams	20.19	12.32	21.15	10.89	3.30
Milliequivalents of acid[a]	122.3	59.9	100.0	46.7	11.7
Milliequivalents of carbonyl[b]	26.4	14.7	23.6	14.5	2.4
Weight of acid, considered as acetic, grams	7.34	3.60	6.00	2.80	0.70
Weight of carbonyl, considered as acetaldehyde, grams	1.16	0.65	1.04	0.64	0.11
Weight of water (by difference)	11.69	8.07	14.11	7.45	2.49
Carbonyl oxygen as per cent of O_2 absorbed	0.8	0.6	0.6	0.7	0.1

[a] Acidity determined by titrating an aliquot of the total trap product.
[b] Carbonyl content (volatile aldehydes and ketones) determined on a separate aliquot of the condenser product by the Bryant and Smith procedure (*6*).

Table III. Oxygen Balance in Lubricating Oil Oxidation

Run No.	66	71	75	79	83	97	99	100
Properties of oil oxidized								
Viscosity, centistokes at 100° F.	34.07	31.35	36.05	23.57	36.05	26.30	28.65	38.56
Kinematic viscosity index	111	119	108	120	108	123	114	101
Average molecular weight	399	402	405	360	405	381	382	403
Temperature of oxidation, ° C.	160	160	150	150	160	150	160	160
O. absorbed in 20 hours/250 grams oil, millimoles	309	456	54	648	137	1052	175	87
Absorbed oxygen recovered as:								
H_2O, %	54.2	50.6	58.2	41.9	53.5	40.4	55.0	60.0
CO_2, %	7.4	8.1	5.0	10.4	6.5	10.7	7.6	5.5
CO, %	2.4	2.7	2.0	2.7	2.2	2.6	2.5	2.1
Volatile acids, %	4.4	5.1	1.9	6.1	3.2	6.6	3.9	2.7
Fixed acids[a], %	2.9	3.4	2.7	3.4	2.9	5.0	2.6	2.5
Total precipitable[b], %	1.6	0.9	1.3	3.0	1.6	4.9	2.5	1.9
Saponifiables[a], %	23.0	19.0	28.0	20.6	25.4	19.7	20.0	28.6
Total accounted for	95.9	89.8	99.1	88.1	95.3	89.9	94.1	103.3

[a] In isopentane-treated oil.
[b] Assuming "total isopentane-insoluble" contains 15% oxygen.

reported as milligrams of lacquer gained by the slide. The use of the glass slide also permits an evaluation of the color of the deposit. In this way the color of the lacquer may be differentiated from its quantity. It is sometimes important to be able to make this distinction.

Measurement of Viscosity Change. The viscosity of petroleum oils usually increases upon oxidation in amounts varying from a few per cent to several hundred per cent. There are, of course, exceptions if the oils contain materials that depolymerize under the test conditions. The viscosity increase is to be attributed to the presence of dissolved precipitable and nonprecipitable products of oxidation including the resinous material adsorbable by clay. The amount of viscosity increase is thus a rough measure of the total quantity of such oxidation products.

Viscosity measurements were made on both the clarified and isopentane-treated products using modified Ostwald pipets to indicate what portion of the increase was due to precipitated bodies. The values were determined at 37.78° C. (100° F), since the percentage change is greater at this temperature than at 98.89° C. (210° F.), and small increases are more apparent. No viscosity measurements were made on the oxidized oils previous to clarification if they contained particles of undissolved material, since such particles clog the viscometer capillaries and make such measurements erroneous.

Determination of Fixed Acidity. Fixed acids are identified as the acids which remain in the oil on completion of the run. It is desirable to differentiate and classify these separately from the volatile acids of low molecular weight. They may include a variety of materials: precipitable acids, possibly phenols, hydroxy acids, lactones, dicarboxylic acids of low molecular weight (if present), etc.

Samples of both the untreated oxidized oil and the isopentane-treated product were titrated for fixed acidity. The neutralization number (milligrams of potassium hydroxide equivalent to 1 gram of the oil) of the oxidized product was calculated, and is the value listed in Table V while the acidity of the isopentane-treated sample was used in calculating the distribution of the oxygen to fixed acids. The latter value therefore does not include oxygen present as fixed acids in the precipitable materials, since it is already thus accounted for.

The A. S. T. M. procedure (*1*) was deemed unsatisfactory for routine determinations in this work and some effort was spent on an electrometric titration procedure described previously (*13*). However, a simpler and quite useful method was devised based on a suggestion of Burshtein (*7*) that in a dark-colored solution an end point might be detected by the appearance or disappearance of fluorescence. Specifically, it was recommended that methyl orange or methyl red could be used to mask the fluorescence of fluorescein when present in their more highly colored forms in acid solution. When the solution is made alkaline the masking effect is lost and the normal character of the fluorescein is apparent. The titration procedure developed on this basis is outlined below.

Solvent. A 50:50 mixture by volume of *n*-butyl alcohol and toluene.

Base. Sodium is added to *n*-butyl alcohol to produce an approximately 0.1 *N* solution of sodium butylate. This is standardized against benzoic acid or potassium acid phthalate using phenolphthalein in water solution. A 10-ml. buret calibrated in 0.05-ml. divisions is employed, and the reagent is protected from carbon dioxide by a tube filled with solid potassium hydroxide.

Indicator. The methyl red solution is prepared by dissolving 0.1 per cent methyl red in the butanol-toluene solvent. The fluorescein solution is made up to a strength of 0.1 per cent in ethyl alcohol. The addition of a few drops of alcoholic potassium hydroxide aids in dissolving the fluorescein.

Procedure. A 4-gram sample (if an acid number higher than 10 is anticipated, a smaller sample is taken) of used or oxidized oil is dissolved in 50 cc. of the solvent. A 500-cc. Erlenmeyer flask is a suitable titrating vessel. As indicators, 2 to 4 cc. of the methyl red solution and 0.2 to 0.3 cc. of the fluorescein solution are then added.

Table IV. Physical Properties of a Group of Commercial Light Oils

Commercial Oil Number	1	6	7	9	10	14	17
Kinematic Viscosity							
Centistokes at 100° F.	39.46	49.50	35.68	36.13	36.99	35.60	78.95
Centistokes at 210° F.	6.07	7.08	5.79	5.48	5.67	5.49	9.64
Saybolt viscosity							
Seconds at 100° F.	183.2	229	166.3	168.3	172.2	165.9	364.7
Seconds at 210° F.	46.0	49.3	44.2	44.2	44.8	44.2	58.0
Kinematic viscosity index	109	110	116	96	102	100	108
Gravity index	110	105	108	96	101	103	109
Gravity, ° A. P. I.	32.7	30.9	32.7	30.1	31.0	31.6	30.9
Viscosity-gravity constant	0.803	0.812	0.805	0.823	0.815	0.812	0.804
Waterman analysis							
Density, 20°/4° C.	0.8580	0.8683	0.8580	0.8721	0.8669	0.8636	0.8678
Refractive index, n_D^{20}	1.4760	1.4824	1.4754	1.4810	1.4761	1.4761	1.4814
Specific refraction	0.3288	0.3286	0.3284	0.3262	0.3255	0.3267	0.3282
Aniline point, ° C.	110.4	105.4	106.8	100.2	106.2	105.6	115.9
Per cent aromatic rings	3	8	5	5	1	2	4
Per cent naphthene rings	15	14	14	16	30	24	15
Per cent paraffin rings	82	78	81	79	69	74	81
Rings per molecule	1.3	1.6	1.3	2.0	2.1	1.7	1.6
Calcd. molecular weight	419	447	414	391	402	391	513
In formula C_nH_{2n+x}							
n	30.0	32.3	29.7	28.2	29.2	28.0	36.8
x	−1.8	−4.6	−2.5	−3.6	−6.9	−2.1	−3.0

TABLE V. OXIDATION CHARACTERISTICS OF A GROUP OF COMMERCIAL OILS

[250 grams oxidized at 170° C. (338° F.) for 20 hours]

Commercial Oil Sample Number	1	6	7	9	10	14	17
Run number	126[a]	130	138	150	151	246	149
Average partial pressure O_2, mm. of Hg	663	675	676	696	695	695	679
Millimoles O_2 absorbed per 250 grams of oil	587	141	203	85	350	68	192
Distribution of absorbed oxygen							
Per cent to H_2O	41.9	53.0	51.0	59.5	50.2	64.6	46.4
Per cent to CO_2	9.1	6.3	7.8	4.5	10.3	5.3	5.4
Per cent to CO	2.2	2.2	2.4	2.1	2.6	1.9	2.1
Per cent to volatile acids	7.8	3.6	4.4	2.4	4.6	2.3	4.3
Per cent to fixed acids	3.3	2.8	2.6	3.2	2.7	4.0	3.5
Per cent to total isopentane-insoluble[b]	2.0	4.2	1.3	5.2	3.8	2.9	2.2
Total thus accounted for	66.3	72.1	69.5	76.9	74.2	S1.0	63.7
Neutralization number of oxidized oil[c]	6.7	1.1	1.9	0.8	3.6	0.6	2.2
Milliequivalents volatile acid per 250 grams of oil	45.7	5.1	8.9	2.0	16.2	1.6	8.3
Precipitable oxidation products:							
A. Total isopentane-insoluble, wt. per cent	1.01	0.50	0.23	0.37	1.11	0.17	0.36
B. Oil-soluble, isopentane-insoluble, wt. per cent	1.01	0.10	0.02	0.01	0.27	0.01	0.10
C. Oil-insoluble (A-B), wt. per cent	0.00	0.40	0.21	0.36	0.84	0.16	0.26
D. Milligrams of lacquer on 3 × 1 inch slide	0.8	1.5	0.9	1.5	5.5	0.09	0.6
Per cent increase in viscosity at 100° F.							
Clarified oil	62.7	12.9	17.7	8.1	22.2	5.7	19.4
Isopentane-treated oil	55.5	12.8	..	7.5	22.0	6.1	18.7

[a] Oxidized at 160° C. (320° F.). [b] Assuming precipitate contains 15% oxygen. [c] Milligrams KOH per gram oil.

The base is added carefully from the buret in the usual way. On the acid side of the neutral point the methyl red masks the green fluorescence which develops strongly and sharply when the solution becomes alkaline. Observation of the end point is aided by illuminating the titrating vessel strongly from the side and using a dark background to reduce transmitted light.

An operator acquainted with the technique is able to titrate stearic acid samples in oil within ±5 per cent of the known value. Checks of a similar order are obtained with the A. S. T. M. procedure. Where the latter fails owing to the presence of large quantities of insoluble bodies or the formation of opaque emulsions, which occur with highly oxidized oils, this titration value may be different from the A. S. T. M. value.

The chief advantages of this procedure, in spite of its indeterminate accuracy and lack of precision, are its simplicity and speed and the effectiveness of the solvent. Thus almost any amount of insolubles will be dissolved and the solvent is completely miscible with the oil so that only one phase is present during titration and no emulsification is possible. The fluorescein method works best for dark-colored oils. A variation tried for light-colored oils of low acidity was the use of an ethanol-toluene solvent and the color change of either bromothymol blue or metacresol purple, without fluorescein. This is satisfactory, provided the oil contributes little or no interfering color.

DETERMINATION OF OTHER OXYGENATED MATERIALS: THE OXYGEN BALANCE. Determination of the volatile and nonvolatile oxidation products indicated above does not account completely for the oxygen which has reacted with the oil. In addition to fixed acids in isopentane-treated oils, considerable quantities of other oxygenated materials are present. Peroxides are found in very small quantities, if at all. Nonvolatile carbonyl compounds such as aldehydes or ketones appear to be present, but are very difficult to determine. The bulk of the remaining oxygen can usually be accounted for as saponifiable material. This may be chiefly esters and anhydrides, but may also include some lactones, phenols, etc.

Saponifiables can be estimated roughly by a modification of the fluorescein acidity titration. A fairly satisfactory method was devised which involved refluxing with 50 to 100 per cent excess alkali in the butanol-toluene solvent for 2 hours, and titration of the excess of alkali using the fluorescein–methyl red indicator.

When saponifiables are considered entirely as esters, and this oxygen content is included in the total balance, 90 to 100 per cent of the reacting oxygen can be accounted for. This is shown for a series of oils oxidized to varying degrees in Table III. Arbitrarily, a value of 15 per cent oxygen present in the "total isopentane precipitate" is used in calculating the value given in this table. It may be seen in Table VIII that this is a reasonable average for such materials. The values for fixed acids and saponifiables include only those materials which were present in the isopentane-treated oxidation product.

Oxidation of Commercial Oil Samples

As an illustration of the application of this procedure, the oxidation characteristics of a group of seven light automotive lubricants will be described. These were oils of S. A. E. 10-W grade (except one which was S. A. E. 20-W) purchased in the open market between August, 1939, and May, 1940. Certain physical properties of these oils are listed in Table IV. In a study of the chemistry of oxidation such as this, it is desirable to have as complete an understanding as it is possible to obtain of the chemical nature of the material being oxidized. This is aided by application of the hydrocarbon-type analysis

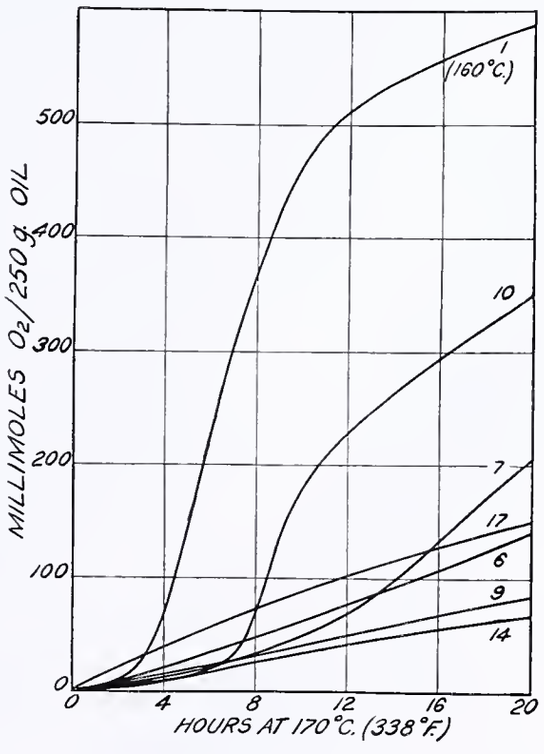


FIGURE 4. OXYGEN ABSORPTION OF A GROUP OF COMMERCIAL OILS
Numbers refer to oils in Table V

Table VI. Molecular Weights of Precipitable Materials Obtained by Laboratory Oxidation

(Molecular weight values were determined cryoscopically using naphthalene as solvent)

Type of Product	Run No.	Molecular Weight
Total isopentane-insoluble	4–8	1650
	21	1530
	32	1280
	51	970
	62	1880
	63	1560
	28	594
Oil-insolubles	13	1680
	24	1480
Oil-soluble isopentane-insolubles	14–17	1510

method of Waterman (*18*), which is based on viscosity, aniline point, refractive index, and density. Average molecular weights were calculated from the viscosity data using the chart of Keith and Roess (*10*).

The absorption curves for these oils are given in Figure 4 and the characteristics of their oxidation in Table V. Because these are commercial products whose source and preparation are unknown, no discussion can be given of the significance of their oxidation behavior in terms of their composition. It will be sufficient to point out the wide variations in oxygen stability which exist among such a group of lubricants. Some exhibit so-called "induction periods" of varying lengths preceding periods of rapid oxidation. Their cleanliness characteristics show similar variations in terms of the amount of insolubles and lacquer produced.

As seen from Tables III and V, the proportion of volatile to fixed or nonvolatile acids is not constant for all oils; in some oils volatile acids predominate over nonvolatile acids while with other oils the reverse is true. If such volatile acids remain in a crankcase, particularly corrosive conditions might result. It may be that discrepancies in correlating service and laboratory corrosion tests are explainable, in part, by the variations in acid types even though the total acidity is the same.

Nature of Precipitable Oxidation Products

The most obscure products of oil deterioration are the solid or semisolid materials which separate from the oil on oxidation—i. e., insolubles and lacquer. It can be assumed that they represent products of oxidation of the hydrocarbon molecules with or without subsequent polymerization and condensation.

Precipitables are light- to dark-brown powders which may be of two types, depending on the nature of the oil from which they are obtained. From relatively aromatic-free oils, such as those produced by exhaustive extraction or heavy acid or clay treatment, the precipitate secured may be a low-melting material of about 30° to 100° C. melting point, soluble in the oxidized oil, and rich in oxygen. Some such oils contain as high as 12 per cent of oil-soluble, isopentane-insoluble substances. Examples are the total isopentane-insoluble samples from run 28, Table VI, and run 52, Table VII. It is probable that the molecular weight of this material is not much higher than that of the parent oil, indicating that condensation and polymerization are not important processes in the formation of this type of material. Another type of precipitable oxidation product is produced from less highly refined oils or from solvent-treating extracts. This is a fine, light powder, usually dark brown in color, and not readily melted, but completely soluble in chloroform or benzene. A sample of such a material was heated to over 200° C. without fusion. The formation of this type of precipitable product is undoubtedly accompanied by condensation and polymerization, since the molecular weight of these materials lies in the range 1000 to 2000 when derived from oils whose molecular weight is 300 to 500, as the data in Table VI show.

Table VII shows the oxygen content of such samples to be fairly high. Of the two types of precipitates, the low-melting material from the oxidation of highly refined or aromatic-free oils has the higher percentage of oxygen which may be greater than 20 per cent as shown by the data from runs 49 and 52 in Table VII. Precipitables from extracts or aromatic-containing oils may contain 13 to 17 per cent of oxygen.

The determination of oil-insolubles is probably of much more significance in predicting engine dirtiness than is that of total isopentane-insolubles, but the latter has a distinct value in indicating the amount of nonfluid oxidized materials which have been produced. The actual total amount of such nonfluid products probably cannot be determined by isopentane precipitation. A precipitant of lower molecular weight would undoubtedly yield a larger deposit. The coloring power of the precipitable product from the oxidation of aromatic- and paraffinic-type oils is quite different. Some aromatic-free fractions have been observed to oxidize to yield as much as 10 per cent of oil-soluble isopentane-insolubles when it was still possible to see through a 1.25-cm. (0.5-inch) layer of the very dark red oxidized oil. Oxidation of such oils at temperatures above 150° C. (302° F.) appears to yield a darker product, however. On the other hand, oils containing aromatic rings oxidized to produce oil-insolubles. In the early stages of their oxidation they become black, even previous to the precipitation of more than 0.1 per cent of material. Unless these oils are oxidized to a high extent, however, there is normally less than 1 per cent of oil-soluble isopentane-insolubles present.

The precipitable bodies have in part the character of high molecular weight acids. Samples from the oxidation of an acetone extract from a distillate oil (run 24) were titrated by the fluorescein indicator procedure. The average molecular weight of the extract before oxidation was 391. The oil-insolubles had a neutralization number of 56 (milligrams of potassium hydroxide per gram of oil) and the oil-soluble isopentane-insolubles had a neutralization number of 38. The molecular weight of the first sample was 1480 (Table VI) and with an acidity of this magnitude there is indicated an average of about 1.5 carboxyl (or acid) groups per molecule of insolubles. Assuming the same value for the molecular weight of the oil-soluble material, the latter would have about one carboxyl group per molecule. Use of these data permit calculation of an average empirical formula for the insolubles. This is:

$$C_{96}H_{92}O_{10}S_{0.3}(CO_2H)_{1.5}$$

Table VII. Elementary Analyses of Precipitable Bodies Obtained by Laboratory Oxidation

Type of Product	From Run No.	Composition (Corrected for Residue, if Any) Carbon %	Hydrogen %	Oxygen[a] %	Sulfur %
Total isopentane-insoluble	4–8	76.56	6.90	16.12	0.42
Same, analysis by another laboratory	4–8	76.39	7.02	16.17	..
Total isopentane-insoluble	52	69.57	9.27	21.08	0.08
Oil-insolubles	13	77.47	7.34	14.83	0.36
Oil-insolubles	24	79.01	6.31	14.08	0.60
Oil-insolubles	49	69.87	7.78	22.35	..
Oil-insolubles	13	77.54	8.10	14.36	..
Oil-insolubles	39	77.93	8.36	13.71	..
Lacquer	11, 12, 13	72.34	6.63	17.39	0.35[b]
Same, analysis by another laboratory	11, 12, 13	72.34	6.63	17.39	0.35
Lacquer, $CHCl_3$-soluble fraction	25, 26, 29	75.07	7.36	17.57	..
Lacquer, $CHCl_3$-insoluble fraction	25, 26, 29	70.71	5.97	23.22	..

[a] By difference, includes sulfur unless latter was determined.
[b] S determined on lacquer composite from runs 18 to 22.

It is possible to study the amount of dehydrogenation and polymerization involved in formation of precipitable materials in the oxidation of a distillate stock by applying data such as the foregoing. The oil was oxidized at an average oxygen partial pressure of 655 mm. of mercury for 50 hours at 170° C. (338° F.). In run 19, 250 grams of oil absorbed 8.74 liters of oxygen (corrected to 0° C. and 760 mm. of mercury), and in oxidizing produced 7.91 grams of water (not corrected for volatile acids) and 7.63 grams of total isopentane-insolubles. From Table VI the latter material has a molecular weight of 1600 and an ultimate analysis of 77 per cent carbon, 7 per cent hydrogen, and 16 per cent oxygen. Waterman hydrocarbon-type analysis indicates that the original oil had a composition approximately corresponding to $C_{29}H_{54}$, and a molecular weight of 402.

Composition of total isopentane-insolubles:

$$\text{Carbon: } \frac{77}{100} \times \frac{1600}{12} = 103$$

$$\text{Hydrogen: } \frac{7}{100} \times \frac{1600}{1} = 112$$

$$\text{Oxygen: } \frac{16}{100} \times \frac{1600}{16} = 16$$

Formula: $C_{103}H_{112}O_{16}$

$$\text{Milligram-molecules of insolubles} = \frac{7.63}{1600} \times 1000 = 4.8$$

Assuming that this material is produced by condensation of oil molecules without loss of carbon, although this may not be at all correct, a roughly balanced equation may be written for the formation of the precipitable material:

$$17C_{29}H_{54} + 172O_2 = 4.8C_{103}H_{112}O_{16} + 190H_2O$$

This equation takes no account of the production of carbon monoxide or dioxide, and indicates a fractional number—namely, about 3.5—of oil molecules reacting to produce one molecule of insolubles. Such processes as decarboxylation and partial combustion of side chains, which undoubtedly take place, are wholly ignored.

Continuing on the above basis, the proportion of the oil molecules which have entered into the formation of precipitables may be estimated:

$$\frac{17}{(250/402)} \times 100 = 2.7 \text{ per cent}$$

The proportion of the water formed which is accounted for by this reaction may also be approximated:

$$\frac{190 \times 18}{7.91 \times 1000} \times 100 = 43 \text{ per cent}$$

Similarly, the oxygen involved out of the total consumption of 8.74 liters is:

$$\frac{172}{(8.74/0.0224)} \times 100 = 44 \text{ per cent}$$

Referring again to Table VII it may be noted that a small amount of sulfur is included in the composition of certain typical precipitable materials. There is considerably less than enough to account for one atom of sulfur to each molecule of insolubles, so it appears improbable that sulfur is necessarily associated with the formation of precipitables. The sulfur content of any one of the samples reflects the sulfur content of the original oil—that is, it is highest from the extract (run 24), next in amount from the original unextracted oil (runs 13 and 48), and lowest from the highly refined oil (run 52). In each case, though, the proportion of sulfur in the precipitable material is of the order of two or three times as great as in the original oil.

Lacquer as produced by laboratory oxidation is a dark-brown powder relatively insoluble in benzene, partially soluble in chloroform, and somewhat soluble in pyridine. Partial chloroform solubility is illustrated by the treatment of the composite lacquer fraction from runs 25, 26, and 29. This material resulted from the oxidation of a distillate stock and averaged 0.089 per cent of the oil charged. Thirty-four per cent of the lacquer was chloroform-insoluble corresponding to 0.030 per cent of the oil. Carbon-hydrogen determinations were made on both the chloroform-soluble and -insoluble fractions, and are listed in Table VII. The very high oxygen content (23.22 per cent) of the chloroform-insoluble fraction is particularly notable. The insolubility of the lacquer prevents an accurate determination of its molecular weight. One attempt at molecular weight estimation using naphthalene as the cryoscopic solvent yielded a value of about 5000, though it was obvious to the operator that the lacquer was not completely dissolved. Partial solubility gives an apparent molecular weight higher than the actual value, since the freezing point is depressed only in proportion to the number of moles dissolved.

Table VIII. Elementary Analyses of Precipitables Obtained from Engine Tests

Source and Type of Product	Composition (Corrected for Residue, if Any) Carbon %	Hydrogen %	Oxygen %	Residue, Weight Per cent
Samples from Chevrolet engine tests				
Oil-insolubles, valve cover plate	75.34	7.63	17.03	4.14
Piston lacquer removed by pyridine wash	73.46	7.88	18.66	0.92
Same, another sample	77.00	7.72	15.28	2.05
Samples from Lauson engine tests				
Piston lacquer from fired engines, Test S-70	75.64	9.42	14.94	7.14
Piston lacquer from motored engines, Test S-72	75.97	8.50	15.53	6.52
Material from automatic transmission				
Lacquer deposit on valves (deoiled by naphtha washing)	75.22	7.99	16.79	26.96

It is of interest in this connection to compare the composition of insolubles and lacquer obtained from engine tests with that of similar materials from laboratory oxidation. Table VIII summarizes the available data on engine products.

The lacquer sample from the Chevrolet engine was obtained by first washing the piston skirt with isopentane to remove oil, then washing a portion of the area with pyridine. The latter solvent removed the lacquer fairly readily, requiring a little rubbing in some portions. Only a portion of the piston area was so treated, amounting to about 110 sq. cm. (6.7 sq. inches) from each of two pistons. The total piston skirt area which acquired a lacquer deposit of this sort was estimated to be about 1100 sq. cm. (67 sq. inches) per engine (six pistons). Since the weight of the deposit was 0.272 gram, the lacquer represents $\frac{5 \times 0.272}{6 \times 950 \times 0.88} \times 100 = 0.027$ weight per cent of an assumed 6-quart charge of oil to the engine. The lacquer deposition on the cylinder walls, etc., is not included in this estimate.

The average thickness of the lacquer deposit can be estimated, assuming the density is 1.0:

$$\text{Thickness, } \frac{0.272}{1 \times 220} = 0.0012 \text{ cm. or } 0.0005 \text{ inch}$$

Similar lacquers were obtained from one-cylinder Lauson engines operated both with and without combustion taking place in the cylinder. In the "unfired" or "motored" runs the engines were driven by electric motors and were effectively just air compressors.

The material from the fired runs was dark brown or black and required pyridine and a brush to remove it. The lacquer from the motored engines more nearly resembled materials produced by laboratory oxidation, and most of it could be brushed off the

piston surface without the use of a solvent, after removing the oil with isopentane.

The similarity in elementary composition of the laboratory and engine test materials is evident on comparing Tables VII and VIII. Engine lacquer samples appear to be somewhat lower in oxygen content than the laboratory samples. A point of resemblance between laboratory and engine test lacquer samples is in partial solubility in chloroform. Most of the engine test samples include a certain amount of inorganic residue, which may be in whole or in part dust, or iron or aluminum resulting from engine wear. A nonleaded gasoline was used as the fuel. The hard lacquer sample from the fired Lauson engines, however, had been dissolved in pyridine and the solution filtered before removing the solvent, so that the residue in this case may represent iron or aluminum which has combined with acidic groups in the lacquer to form a salt. Because of the very minute quantity of sample available, the composition of this residue was not determined.

In subsequent papers the oxidation of oil fractions differing in molecular type and weight will be reported.

Acknowledgment

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PRESENTED before the Division of Petroleum Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Adjustable Mercury-Filled Metal Thermoregulator

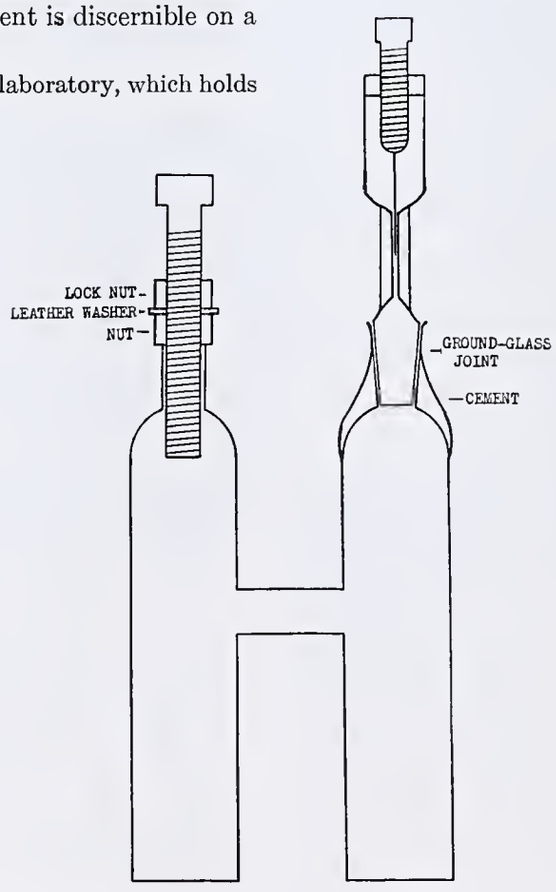
FRANK PADGITT, Louisiana State University, University, La.

A METAL, mercury-filled regulator for thermostats which may be made by any welder at the cost of only a few dollars has been in use in this laboratory for nearly a year with satisfactory results. It is capable, when used with the proper supporting units (*1–4*)—vacuum-tube relay, efficient stirring, etc.—of maintaining a temperature of ±0.001° C. for considerable periods of time. In fact, for short periods of an hour or two no movement is discernible on a Beckman thermometer.

The design of the regulator used in this laboratory, which holds about 130 ml. of mercury, is shown in the figure. It was made from thin-walled automobile tail pipe, which may be obtained at any automobile supply house. The smaller pipe is ordinary steel tubing. It must be welded with steel rod and so constructed that air bubbles will not be trapped within.

The method used to avoid removing or adding mercury when changing from one temperature to another is a modification of that of Parks (*5*), who used a nickel rod carrying a rubber plunger fitting tightly in a glass tube, and of Yee and Davis (*6*), who used an Invar steel plunger in a Jena KPG tube of very uniform bore. The adjustment device consists of a 0.938 × 10 cm. (0.375 × 4 inch) machine bolt which can be screwed in or out of the regulator through the nut welded to one arm. The assembly is made leakproof by a lock nut which may be tightened upon a thin leather washer between it and the nut welded to the regulator. Enough mercury is displaced by screwing the bolt in or out to change the temperature over a range of about 90° C.

On the other arm of the regulator a standard tapered joint is sealed to the metal with cement. (A satisfactory cement for this purpose in oil baths is Sauereisen Insa-Lute No. 1 made by Sauereisen Cements Company, Pittsburgh, Penna., as it neither softens nor becomes brittle with change of temperature. When used in a water bath, the surface of this cement must be waterproofed.) A piece of 0.7-mm. capillary tubing is sealed to a ground-glass joint and connection made to the regulator in this manner. This allows the capillary to be readily removed and cleaned. Final adjustment of the temperature is made in the usual manner.



This regulator possesses the advantages of rapid heat transfer through the thin walls of the tail pipe, extreme ruggedness, and a simple effective adjustment for changing the temperature over wide ranges.

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Editor's Note. Some time ago several notes appeared in the NEWS EDITION under the general title "Reminders". They made no claim to priority or originality, but reintroduced good ideas and sound practices already known to the older generation of chemists. More recently the ANALYTICAL EDITION has carried a note or two directing attention to procedures or equipment already known and used by some of the profession though not readily accessible in print. This has prompted submission of the two printed here.

Simple Vacuum Still for Purification of a Single Substance or Recovery of a Single Fraction

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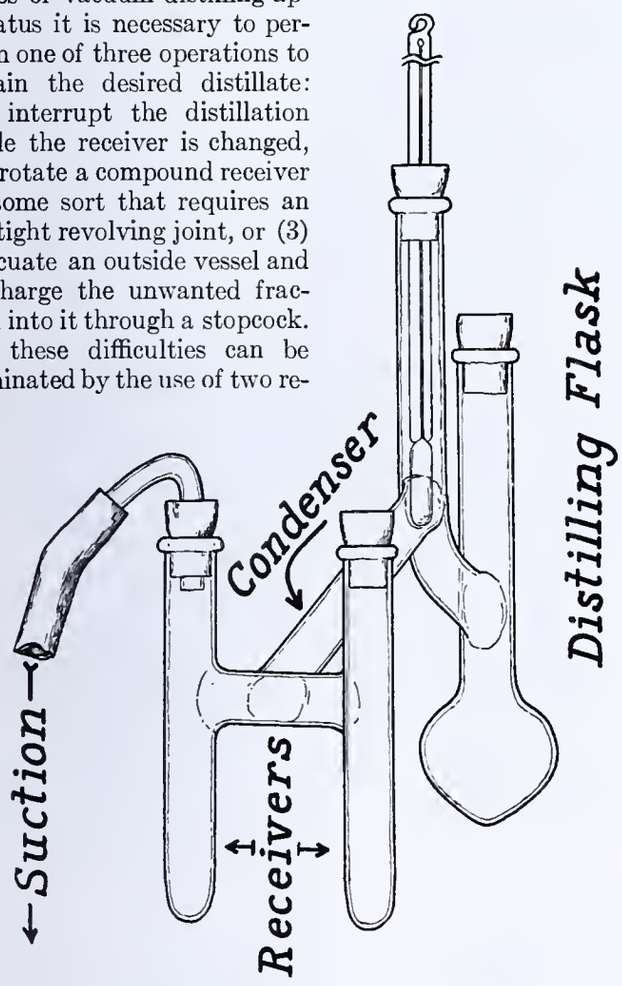
WHEN the purpose of a distillation is to obtain a substance in the distillate free from impurities from which it is to be separated, it is necessary to save only one fraction. Usually this fraction is preceded by a more volatile fraction or fractions which must be rejected. In the conventional types of vacuum distilling apparatus it is necessary to perform one of three operations to obtain the desired distillate: (1) interrupt the distillation while the receiver is changed, (2) rotate a compound receiver of some sort that requires an air-tight revolving joint, or (3) evacuate an outside vessel and discharge the unwanted fraction into it through a stopcock. All these difficulties can be eliminated by the use of two receivers connected with the lower end of the condenser by a T-shaped tube. The distillate is directed into one or the other of the receivers by tipping the entire apparatus, thus avoiding the necessity for flexible or rotating joints or glass stopcocks.



Similar T-shaped connections could be used in a variety of apparatus. A T-shaped adapter could be connected to two flasks and to the condenser through rubber stoppers. Such a device would permit more latitude in the selection of various types of air- or water-cooled condensers, receivers, still heads, and distilling flasks; and the flexibility of the stoppers would decrease the danger of breakage. Standard taper joints could be used where advantageous. The one-piece construction shown in the figure has the advantage of eliminating several points where leakage could occur and where contamination from rubber might enter, especially in high-temperature distillations. The still shown is presented both for its own utility and as an example of the use of the T-connection.

The apparatus involves little or no innovation in principle or design.

The still is supported in a clamp or clamps having asbestos-covered jaws, in such a way that it will not slip but can easily be rotated around the long axis of the condenser. During the first part of the distillation the entire apparatus is tipped back, so that the more volatile fractions to be rejected are delivered into the receiver away from the operator. As soon as the desired fraction starts to come over, it is tipped forward and this fraction is collected in the clean receiver. At the end of the collection of this fraction, the apparatus is quickly tipped back again to reject any heavier distillate that may come over before the distillation can be stopped. At any time during the distillation of the desired fraction the still may be tipped back momentarily to reject contaminating material which may splash over, where this can be detected. Unwanted fractions, both above and below, are thus collected in one receiver, and the fraction to be saved is in the other.

The apparatus illustrated proved highly satisfactory for the purification of a few milliliters of oleic acid. Because of violent bubbling in the flask and the abrupt appearance of a brownish contaminant, considerable agility was required to save the clear colorless distillate that had already been obtained, and it would have been impossible to save it if more than 2 seconds had been required to change receivers.

Removal of Stopcock Grease from Buret Tips

C. B. WHITE AND R. C. MCGLENN

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A VERY common hindrance to the analytical chemist's titrations is the plugging or partial stoppage of the buret tip when too much stopcock grease has been used. The writers have seen several methods of removal employed, including hot water, pieces of wire, and the warming of the tip with a match. However, the use of carbon tetrachloride as a grease solvent gets the best results.

By leaving the stopcock in the open position and dipping the tip, up to the stopcock itself, several times in a small beaker of carbon tetrachloride, the last vestiges of grease will be removed. If the tip is not sufficiently open to permit the entrance of the carbon tetrachloride, a fine piece of wire thrust through the grease first will give the solvent the opportunity to accomplish its task. This carbon tetrachloride method eliminates the possibility of breaking the buret by using hot water or a flame, and achieves what is wanted better than either of these methods.

Chemical Determination of Nicotinic Acid

AARON ARNOLD, C. B. SCHREFFLER, AND S. T. LIPSIUS
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THE role of nicotinic acid in the prevention or cure of black tongue in dogs (*5*) and pellagra in humans (*14*) has stimulated studies on methods for the quantitative determination of this compound. The chemical methods are based on its reaction with 2,4-dinitrochlorobenzene (*7*, *17*) or with cyanogen bromide and aniline (*9*, *10*, *11*, *16*) or *p*-methylaminophenol sulfate (*2*, *3*, *12*) as color reagents. Recently, Harris and Raymond (*6*) have reported considerable success for the evaluation of nicotinic acid in urine with *p*-aminoacetophenone as the color reagent. Since the authors have been engaged in utilizing rapid laboratory methods for the determination of the factors of the vitamin B complex, they have adapted this latter method for the evaluation of nicotinic acid in natural and concentrated source materials in a manner somewhat different from the procedure developed by Kodicek (*8*). They believe the general interest in the antipellagra factor warrants recording their method and results at this time.

Experimental

PREPARATION OF EXTRACT. An amount of finely ground or minced sample, designed to supply about 0.4 mg. of nicotinic acid, is suspended in 75 cc. of water in a centrifuge bottle and autoclaved for 15 minutes at 10,500 kg. per sq. meter (15 pounds per sq. inch). This step is similar to the one used in the preparation of riboflavin extracts, which has been found fully satisfactory for adequate extraction (*1*, *13*). After cooling, the bottles are centrifuged and the supernatant liquid is decanted into a 125-cc. Erlenmeyer flask. The residue is taken up with enough water to bring the total amount of extract up to about 80 cc., centrifuged, and decanted for addition to the first extract.

Some nicotinic acid–rich materials do not require this preliminary extraction treatment, but yield reliable values when treated directly in the same way as the extracts. A few such cases are shown in Table I.

The combined extracts of the nicotinic acid–rich materials, made up to a volume of about 80 cc. with water, are made alkaline with 5 cc. of 20 per cent sodium hydroxide solution and are heated for half an hour on a steam bath or hot plate to liberate nicotinic acid from its amide. Then 2 cc. of 4 per cent sodium bicarbonate solution are added to the cooled solution to aid in adjusting the pH, 1.5 cc. of concentrated hydrochloric acid are added, and the solution is brought to a pH of 6.0 to 6.2 with hydrochloric acid (10 per cent). The solution is transferred to a 100-cc. volumetric flask and diluted to the mark with water.

TREATMENT OF EXTRACT. Aliquots (5 cc.) of the solution containing the unknown are measured into each of four 15-cc. amber glass graduated cylinders (Otto R. Greiner Co., Newark, N. J., 1.0-cc. graduations). These are diluted with 5 cc. of pH 6.0 potassium dihydrogen phosphate–sodium hydroxide buffer solution (*4*) to help ensure comparable pH conditions between separate determinations. With nicotinic acid–low materials 10-cc. aliquots are sometimes necessary, in which event the buffer solution is omitted.

Twenty and 40 micrograms of nicotinic acid, as a solution containing 100 micrograms of nicotinic acid per cc., are added to two of the graduates, all the cylinders being then placed in a water bath at 80° C. After 10 minutes, 2.0 cc. of freshly prepared cyanogen bromide (saturated bromine water just decolorized with 10 per cent potassium cyanide) are added to three of the graduates, including those containing the added nicotinic acid, and all four cylinders are held at 80° C. for an additional 4 minutes. The solutions are then rapidly cooled to room temperature and after 4 minutes, 0.2 cc. of *p*-aminoacetophenone solution (10 grams dissolved in 28 cc. of 10 per cent hydrochloric acid and diluted to 100 cc.) is added to each graduate. After mixing the contents, the cylinders are allowed to stand in a darkened place for 15 minutes. After that time, 0.4 cc. of 10 per cent hydrochloric acid is measured with a microburet into each cylinder and the solutions are allowed to stand for an additional 15 minutes in the dark. The volume in each cylinder is then adjusted to 13 cc. with water.

The reaction mixtures are transferred to 25-cc. centrifuge separatory funnels (Pfaltz & Bauer, Inc., New York, N. Y.) containing 15 cc. of ethyl acetate. The funnels are shaken for 5 minutes, though a somewhat shorter period may be equally satisfactory, and the mixtures are allowed to separate, centrifuging for half a minute to clear the

TABLE I. NICOTINIC ACID CONTENT OF SOURCE MATERIALS

Material	*p*-Aminoacetophenone Procedure			Other Methods	
	Amount assayed[a]	Extraction procedure	Direct alkali treatment	Bioassay values (*18*)	Chemically obtained values
	Gram	*Mg. %*	*Mg. %*	*Mg. %*	*Mg. %*
Fresh beef liver	0.125	9.2	9.2	25–27.5	9.3 (*7*) 12.2 (*2*) 18.0 (*9*)
Fresh beef muscle	0.4	2.4, 2.5	...	3.8–10.2	3.8 (*7*) 4.1 (*2*) 4.9 (*9*) 4.0 (*8*)
Ether-extracted peanut meal A	0.1	22	...	....	....
Peanut meal B, 6% fat	0.1	20, 23	...	13 (raw meal)	16.7 (*16*)
Defatted wheat germ A	0.4	3.4	...	....	....
Defatted wheat germ B	0.4	2.9, 3.0	...	Less than 6	2.9–3.9 (*8*)
Brewer's yeast	0.05	41	38	34–93	33.9–36.7 (*3*)
Baker's yeast	0.05	29, 32	...	50	44.6, 45.5 (*3*)
Feed yeast	0.07	34, 37	...	....	....
Rice bran extract	0.0137	....	174, 180	165	....
Rice bran	0.075	28, 29	28, 31	....	....
Rice germ	0.1	15, 16	...	....	....

[a] Expressed in terms of weight of original sample supplied by 5-cc. aliquot used.

emulsion if necessary. The aqueous lower layer is drawn off and discarded. The ethyl acetate layer is clarified by adding about 2 grams of anhydrous sodium sulfate, whereupon it is ready for the colorimetric reading.

Instrument. A recently developed fluorometer (Pfaltz & Bauer) has proved entirely satisfactory for estimating the concentration of the color complex. The characteristics of the instrument rendered it unsuitable for use in direct readings of the unextracted color complex, which was therefore extracted with ethyl acetate as described above.

The authors determined the extinction curve of the nicotinic acid–*p*-aminoacetophenone color complex in this solvent, and noted a marked inflection at 420 mμ which led them to use Corning filters 038 and 511. The filters are placed in the metal tube of the instrument between the capillary mercury arc tube and the cuvette containing the color complex in ethyl acetate. The following instrument settings have been found satisfactory: rheostat setting at 2.0, resistance box setting at 2.0 (coarse), and an iris diaphragm setting at approximately 20. The light intensity will vary with the age of the tube and is adjusted to bring the galvanometer indicator to 0.0 on the extinction scale when a standardizing cuvette with ethyl acetate is in position. The low-sensitivity range of the galvanometer is used for the readings.

Readings and Determination. Twelve cubic centimeters of the clarified ethyl acetate solution are placed in a cuvette and the concentration of the color complex in each of the aliquots is estimated on the basis of the observed extinction values.

The extinction value for the blank (no cyanide added) is subtracted from the values obtained for the aliquots containing 0, 20, and 40 micrograms of added nicotinic acid. The corrected extinction coefficients are plotted against the amounts of added nicotinic acid. The nicotinic acid content of the unknown can then be read from the graph, by extending the line to its intersection with the abscissa, as shown in Figure 1 for two determinations which are discussed below.

In general, the authors prefer to obtain a reading of about 20 micrograms for the aliquot of the unknown. The amount of extract prepared permits four determinations, so that little difficulty is experienced in obtaining an aliquot which will supply approximately the required amount of nicotinic acid.

Recovery of Added Nicotinic Acid. Figure 1, which illustrates the determination of the nicotinic acid content defatted wheat germ, also shows the excellent recovery which may be expected in the determination of the added nicotinic acid. An 8.0-gram sample gave a value of 13.7 micrograms of nicotinic acid per 5-cc. aliquot (curve B), representing (13.7 × 20) 274 micrograms for the 8.0-gram sample, or 3.4 mg. per cent; 4.0 grams of the same wheat germ to which had been added 200 micrograms of nicotinic acid gave a reading of 17.0 micrograms per 5-cc. aliquot (curve A), representing a total of (17.0 × 20) 340 micrograms of nicotinic acid for the sample. This represents a recovery of 102 per cent for the added nicotinic acid.

The extinction coefficient for the blank aliquot (no cyanide added) had a value of 0.044 in the case of curve B and half this figure in the case of curve A. The amount of interfering color was therefore relatively low.

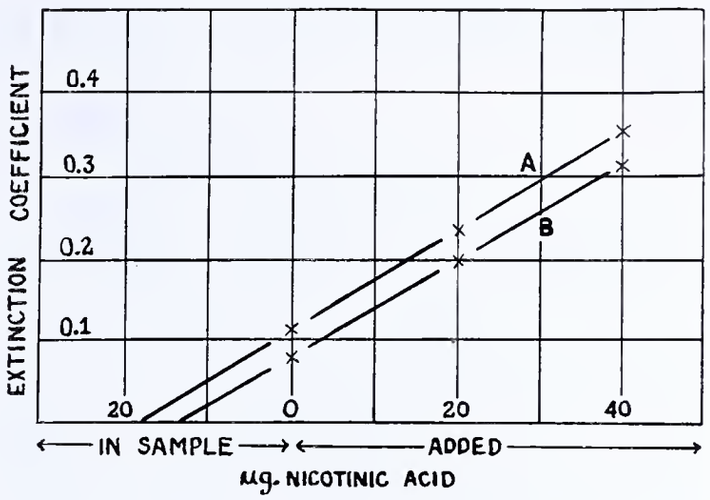

Figure 1. Recovery of Added Nicotinic Acid

A. 4.0 grams of defatted wheat germ + 200 micrograms of nicotinic acid
B. 8.0 grams of defatted wheat germ

Discussion

Data on the nicotinic acid content of various source materials are given in Table I together with those of other investigators. The results of Waisman, Mickelsen, McKibbin, and Elvehjem (*18*) are of particular interest, since they are reported on the basis of bioassays with dogs.

The variation in the values for the nicotinic acid content of fresh beef liver may be due to the variation in the age and nutritive condition of the animal, as indicated by Waisman *et al.* (*18*). The bioassays of fresh beef muscle also indicate a similar variation in nicotinic acid content. It is important to note, however, that the authors' results, the bioassay values, and the figures obtained by other chemical methods are essentially of the same order on comparable materials.

The nicotinic acid values for samples of low-fat peanut meal, 20 to 23 mg. per cent, and the biologically assayed raw peanut meal, 13 mg. per cent, agree when compared on the same basis, since raw peanuts contain approximately 50 per cent fat.

The authors' results with defatted wheat germ, 2.9 to 3.4 mg. per cent nicotinic acid, are in accord with the observation of Waisman *et al.* (*18*) that this dietary supplement cannot be regarded as a good source of the antipellagra factor.

It is interesting to observe that the rice kernel furnishes by-products which are a good source of the antipellagra factor. The germ layer, which contains approximately 15 mg. per cent nicotinic acid, is a much better source of this factor than wheat germ. Rice bran ranks close to yeast as a source of the antipellagra factor and was, in fact, early (*15*) recognized as a source of both nicotinic acid and the antiberiberi vitamin.

The results demonstrate that the method is applicable to a wide variety of materials. From the standpoint of laboratory procedure, it appears simpler than some of the methods submitted by other investigators, while the color reagent proposed by Harris and Raymond (*6*) also appears to be more stable than those previously suggested. The color complex is readily extracted with ethyl acetate and yields, in general, blanks with very little color due to interfering substances.

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Determination of Electrolytic Copper

A Microcerimetric Method

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THE classical Pregl method (*2*) of determining copper by electrolysis from a sulfuric acid bath has been used successfully with certain limitations. In the Pregl method, a platinum electrode is weighed on a microbalance before and after deposition of the copper.

The time factor imposed by microgravimetric procedures is objectionable, even when the microbalance is readily available. Hence, a volumetric method utilizing ceric sulfate oxidation of the copper has been developed in order to facilitate rapid, accurate determinations of the electrolytic copper. During the course of investigation, the Pregl electrolysis technique was modified somewhat in order to ensure complete deposition and provide for a minimum of manipulation.

TABLE I. CHLORIDE EFFECT ON COPPER TITRATION

(1.234 mg. of copper taken)

Chloride Concentration	Copper Found	Error	Chloride Concentration	Copper Found	Error
N	*Mg.*	%	*N*	*Mg.*	%
0.025	1.215	−1.74	1.0	1.232	−0.18
	1.170	−5.2		1.246	+1.10
	1.239	+0.38		1.245	+1.01
0.5	1.233	−0.08		1.226	−0.74
	1.228	−0.56	2.0	1.242	+0.74
	1.235	+0.08		1.237	+0.28

The method described is based on the finding that electrolytic copper can be stoichiometrically oxidized with excess ceric sulfate in 2 *N* sulfuric acid when the proper amount of chloride ion is present. The excess ceric sulfate can then be titrated with Mohr salt, using *o*-phenanthroline ferrous complex as an indicator.

A fine-grained adherent copper deposit may be obtained by electrolysis at 2.0 volts from a 2 *N* sulfuric acid bath when the bath is 0.8 *N* to 1.0 *N* in nitric acid. This concentration of nitric acid prevents interference of other metals when electrolysis is conducted at this voltage.

FIGURE 1

Reagents

Ceric sulfate solution, approximately 0.015 *N*, was prepared according to the instructions of Willard and Young (*5*) modified by Kirk and Tompkins (*1*), and carefully standardized against pure sodium oxalate by the procedure of Smith and Getz (*3*).

Ferrous ammonium sulfate (Mohr salt) solution, approximately 0.015 *N*, was prepared in 0.1 *N* sulfuric acid, and accurately standardized against pure sodium oxalate through ceric sulfate solution. Before standardization, 0.025 *N* ferrous *o*-phenanthroline was added in the proportion of 2 drops to each 5 ml. of solution.

Copper sulfate solution containing approximately 1 mg. of copper per ml. of solution was prepared from selected crystals of copper sulfate. The solution was analyzed by the method of Swift (*4*).

Apparatus

The electrolysis apparatus was essentially that of Pregl (*2*), the most important modification being in the condenser, which was constructed as shown in Figure 1. Experience has shown that if the solution bumps or foams, the Pregl condenser does not have enough condensing surface to prevent some loss of liquid. Introducing the bulb within the electrolysis cell and inserting a capillary, *a*, of 1-mm. bore and 50-mm. length to assist in building up the convection circuit of the condenser water increased the efficiency markedly, as could be readily demonstrated by adding dye solution.

A small funnel and siphon were provided for washing out the electrolyzed solution at the end of the electrolysis. This was performed until the current was essentially zero, as indicated by an ammeter in the electric circuit.

Procedure

Samples of known copper content were placed in the electrolysis cell and the solution was made 2 *N* in sulfuric acid and 1 *N* in nitric acid. The electrodes were inserted, the condenser was put in place, and the solution was heated to boiling. The circuit was closed and electrolysis continued for 8 minutes.

The condenser and flame were removed, a small funnel and siphon were put in place, and distilled water was circulated through the cell until the ammeter reading dropped to zero. The gauze electrode was removed, given a quick rinse in distilled water, and immersed in 10 ml. of the standard ceric sulfate solution made 0.5 *N* in chloride ion. After 2 minutes of stirring with the electrode, this stripping solution was titrated back with the standard Mohr salt solution.

With the proper setup and using two gauze cathodes (one solution being electrolyzed while a titration was being carried out) complete determinations could be made in not more than 20 minutes. Two equivalents of ceric ion were reduced per mole of copper, which was oxidized from the metallic state to cupric ion.

Results

It had been observed that the direct oxidation of electrolytic copper by ceric sulfate alone was not satisfactory, apparently because of a slow rate of oxidation of the metallic copper to cuprous ion, giving an opportunity for air to produce a portion of the oxidation in the acid solution. The addition of chloride ion markedly improved recovery, coinciding with a large increase in rate of the first oxidative step. Since chloride is not rapidly oxidized in the cold by ceric sulfate, this procedure was tested. The most favorable concentration of chloride was studied, the results being given in Table I. Dilute chloride tended to give slightly low and variable recovery, while 1 and 2 *N* concentrations showed the formation of a yellowish-green color, tending to obscure the end point. The most favorable concentration was found to be 0.5 *N*.

TABLE II. COPPER DETERMINATION

Copper Taken	Copper Recovered	Error	Copper Taken	Copper Recovered	Error
Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
1.234	1.233	−0.08		0.496	+0.82
	1.228	−0.56		0.490	+0.41
	1.235	+0.08		0.493	+0.24
	1.233	−0.08	0.243	0.242	−0.41
0.492	0.490	−0.41		0.243	0.00
	0.497	+1.02		0.252	+3.7
	0.487	−1.02		0.242	−0.41

Table II shows the data obtained in determining various quantities of copper from about 0.2 to 1.2 mg., using the method outlined. In all but one case the errors were not greater than 1 per cent, an accuracy which compared satisfactorily with the gravimetric procedure. The time and technical difficulties were definitely reduced over those of the standard micromethod.

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Titan Yellow Qualitative Test for Magnesium

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THE use of titan yellow as a qualitative reagent for magnesium was first proposed by Kolthoff (*6*). Further studies of qualitative applications and interference were made by Barnes (*1*), Eilers (*3*) Eegriwe (*2*), Kolthoff (*7*), van Nieuwenburg (*9*), and Feigl (*4*), and it is reported in Organic Reagents (*10*) and in Tables of Reagents (*5*). However, as the information concerning interference is incomplete and contradictory, this investigation was undertaken to make further study of the interferences of various ions with the magnesium–titan yellow reaction. It was first necessary to study the conditions required for the reaction.

Test-Tube Method

Kolthoff (*6*) used 10 ml. of solution, 0.2 ml. of 0.1 per cent aqueous titan yellow solution, and 0.25 to 0.5 ml. of 4 *N* sodium hydroxide, and reported the detection of 0.0002 mg. per ml. and an increase in intensity of color with an increase in concentration of magnesium. This was found to be true up to a concentration of 1 mg. of magnesium per ml. Further increase in the concentration of magnesium produced a decrease in the color intensity and, at a concentration of 50 mg. per ml. a white precipitate was formed and the solution remained yellow. On the addition of more sodium hydroxide the precipitate became light orange, then pink.

Adding sodium hydroxide slowly from a buret to magnesium–titan yellow solutions, the color was found to change gradually from yellow to orange to red. The exact point was hard to determine, but a pH of about 12.5 was necessary to produce the red color. Reversing this change by the addition of acid, the point of color change, again difficult to detect, was around 11.5.

Hence, concentrated solutions of magnesium fail to give the test unless an excess of sodium hydroxide is added, as all the hydroxide is used to precipitate the magnesium and the pH is too low to give the red color. Therefore, if a white precipitate and no red color are obtained, either an excess of sodium hydroxide should be added or a dilution made and the test repeated. The dilution is preferable, as sodium hydroxide itself at high concentration gives a brownish-orange color that may be confused with the magnesium color.

Spot Plate Method

Feigl (*4*) placed one drop each of solution, 0.1 per cent titan yellow, and 0.1 *N* sodium hydroxide on a spot plate and was able to detect 1.5 micrograms of magnesium at a concentration of 0.03 mg. per ml. Since Kolthoff (*6*, *7*) reported that calcium and barium do not interfere but intensify the color, saturated solutions of calcium and barium hydroxides were substituted for sodium hydroxide. Slightly better color was obtained, barium being preferable, and magnesium was detected at 0.02 mg. per ml. As with the Kolthoff method, the intensity of color increased to a maximum at 1 mg. per ml., then decreased to none at 50 mg. per ml.

Further difficulties were encountered when studying interference by this method, as only a slight lowering of pH prevented the formation of the red color and the concentration of titan yellow was so great that an increase of hydroxide produced a color hard to distinguish from the magnesium color. Various concentrations were tried, and satisfactory results were obtained by the use of 1 drop each of solution, 0.01 per cent titan yellow, and 2 *N* sodium hydroxide. In the absence of magnesium, the color is yellow to taupe and in the presence of magnesium, pink. The difference in color is sufficiently distinct to cause no confusion. Magnesium can be detected at a concentration of 0.01 mg. per ml., the intensity of color increases continuously with increasing concentration of magnesium through 50 mg. per ml., and much interference is eliminated. Titan yellow solutions are more stable at higher concentrations; so 0.1 per cent solutions, which will keep for at least 11 months, may be prepared and dilutions made as needed.

Reaction Paper

In Tables of Reagents (*5*) it is stated that the test may be carried out on reaction paper. No specific directions for its use were found. Tests were made over a wide range of concentrations by both the drop and impregnated paper methods. No conditions could be found that did not give the same results whether magnesium was present or not. This is consistent with Eilers' (*3*) report that he obtained the test with magnesium-free filter paper.

Effect of Other Ions

The cation solutions were chlorides, nitrates, or sulfates and contained 10 mg. of the respective cation per ml. The anion solutions were sodium or potassium salts, except the oxalate which was ammonium, and contained 0.2 gram of salt per 10 ml. of solution. Tests were made by the Kolthoff and the modified spot plate methods on the solution with and without the addition of a magnesium solution containing 0.1 mg. per ml. For the Kolthoff method, 10 ml. of solution and 2 ml. of magnesium were used; for the spot plate, one drop of each.

Results and Discussion

The reaction of titan yellow with magnesium is not specific, but is shown by many cations. As with magnesium, each cation has its characteristic pH of reaction and is further affected by the concentration of the ion and of titan yellow. Silver, mercurous, and mercuric ions react in neutral or slightly acid solution to give a red color with titan yellow if both the cation and titan yellow are of high concentration.

Cadmium, calcium, cobalt, copper, lead, lithium, manganese, and nickel react under the conditions used in testing for magnesium. In the test tube, only the blue copper hydroxide precipitate could be seen, but on the spot plate two precipitates, a blue and a red-lavender, were distinctly visible. All these ions gave red or pink precipitates at 10 mg. per ml., none at 0.1 mg. per ml. Copper, cadmium, cobalt, manganese, and nickel reacted at 1 mg. per ml. while calcium, lead, and lithium did not. It is possible that other cations may react under different conditions—for example, barium was found to react on standing. Some of these that did react were so near the critical pH that slight differences in the pH of initial solutions as well as differences in concentration of solutions used by different investigators may account for some of the contradictory results reported. The intensification of color produced by barium, calcium, and cadmium (*6*, *7*) is probably due to their coprecipitation with magnesium under conditions where they did not precipitate alone.

The following ions, because of their color or that of compounds formed with hydroxide, mask the magnesium–titan yellow color—chromium, copper, ferrous, ferric, mercurous, mercuric, silver, and permanganate.

With the modified spot plate method, aluminum, lanthanum, and zinc prevent the formation of the magnesium–titan yellow red color. With the Kolthoff procedure, aluminum, ammonium, antimony, arsenic, lanthanum, stannous, stannic, and zinc prevent the formation of the magnesium–titan yellow red color. In each case, however, the pH was below 12.5 which is the minimum necessary to produce the red color, and the addition of excess concentrated sodium hydroxide did give the red color. This high concentration of sodium hydroxide, however, is not advisable in testing procedures, as it alone gives a color that can be confused with the magnesium test. Kolthoff (*6*) attributes the interference of tin and aluminum to their adsorption of magnesium. However, regardless of adsorption, the presence of these ions lowers the pH below that necessary to produce a red color.

In general, anions had no effect on the reaction, but tartrate and ferricyanide did decrease the sensitivity and permanganate masked the color. The following ions did not interfere with the test by either the Kolthoff or modified spot

plate method—barium, bismuth, potassium, sodium, strontium, acetate, arsenate, arsenite, borate, bromide, carbonate, chlorate, chloride, chromate, cyanide, cyanate, ferrocyanide, iodide, nitrate, sulfate, sulfite, thiocyanide, and thiosulfate.

Comparison with Other Tests

When carried out under similar conditions, the limit of detection of magnesium by titan yellow, *p*-nitrobenzeneazoresorcinol, or *p*-nitrobenzeneazo-α-naphthol is about the same. There may be a matter of personal preference between a red and a blue color. Titan yellow has the advantage of developing its color rapidly, whereas in dilute solutions the others, especially the resorcinol compound, develop their colors slowly, so that it is necessary to wait 5 or 10 minutes. Mehlig and Johnson (*8*) show that traces of aluminum, barium, calcium, and strontium present through faulty separations will not interfere with the *p*-nitrobenzeneazoresorcinol test, whereas they do interfere with phosphate. The present work shows that the same is true with titan yellow for traces of barium, calcium, and strontium but that aluminum will prevent the test. These color tests are quicker, more easily recognized, and require less solution than the phosphate test.

Summary

The titan yellow test, although not specific for magnesium, is useful and efficient when properly applied. It is suitable for test tube or spot plate but not reaction paper. An improved spot plate procedure is given. A pH of about 12.5 is necessary for the production of the red color. Interference may be caused by ions which undergo a similar reaction, by ions which because of their color or that of compounds formed with hydroxide mask the magnesium–titan yellow color, or by ions which keep the pH too low. Ions which interfere, however, will be removed in the usual qualitative procedure before testing for magnesium and hence cause no trouble.

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A Microphotometer for Spectrochemical Analysis

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THE increasing use of quantitative spectrochemical analysis and the accompanying improvements in the reliability of the sources employed have stimulated interest in microphotometers. The instrument companies have improved old models and developed new ones. In addition, numerous designs have been described in recent literature. This note describes an instrument which, while not so fast nor so accurate as some, is convenient to use and is satisfactory for much work. It is easily assembled from standard equipment, part of which is probably available in many laboratories; hence, the outlay of time and money required for its construction is small.

Apparatus

Figure 1 shows the arrangement. A microscope furnishes a convenient foundation for the instrument. The photographic plate to be measured is mounted on a mechanical stage, thus enabling the observer to bring any spectral line into position quickly and easily. The plate is illuminated by a 32-candlepower single-filament automobile headlight bulb which receives its power from a voltage-regulating transformer. An enlarged image of the spectral line is directed to a horizontal slit in front of a photocell by a right-angle prism mounted on a strip of metal which is hinged near one end and supported by a micrometer screw at the other. The photocell is connected to a wall galvanometer, the scale of which is mounted over the microscope. This scale is not shown in the photograph.

The mechanical stage is easily adapted to hold photographic plates 2 inches (51 mm.) wide. The supports which were designed to hold microscope slides are removed and a plate of 0.125-inch (3-mm.) brass 2.5 × 5 inches (64 × 127 mm.) with a rectangular hole 1.5 × 3.5 inches (38 × 89 mm.) cut in it is screwed on in place of them. The photographic plate (or a piece of 35-mm. motion picture film mounted in a light brass frame) is held on this brass plate with phosphor bronze clips.

FIGURE 1. MICROPHOTOMETER

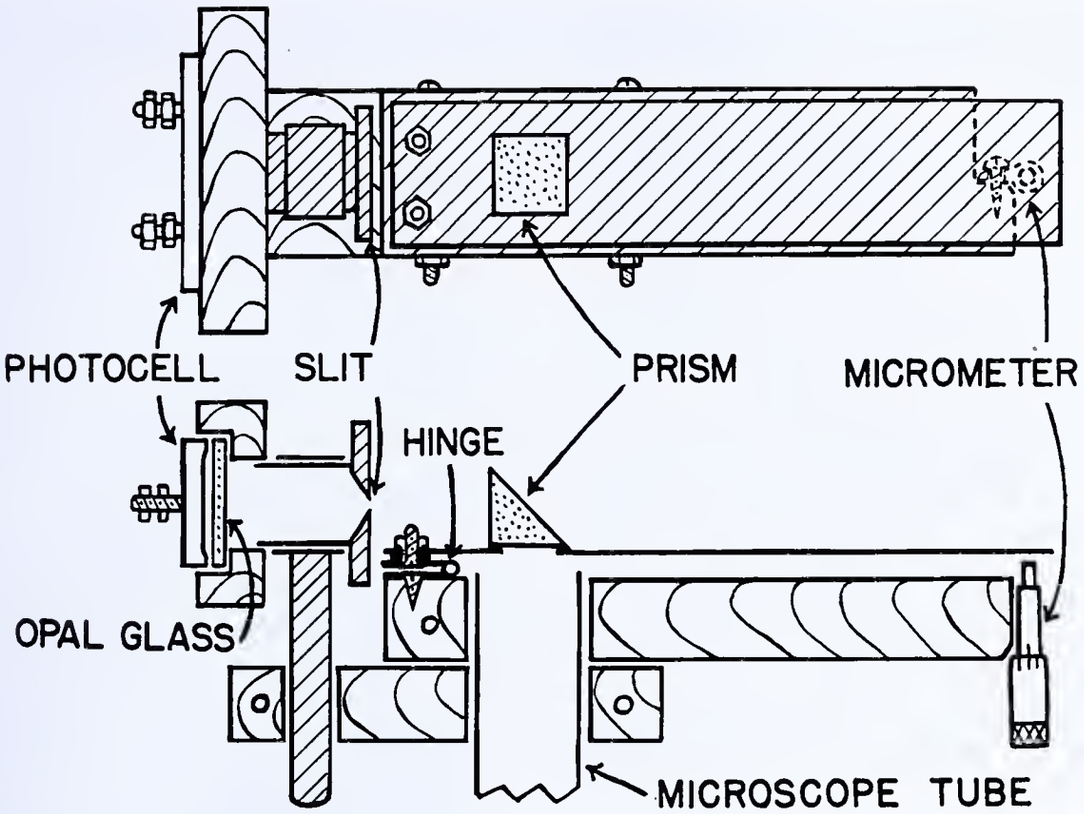

FIGURE 2. DETAILS OF UPPER PART OF MICROPHOTOMETER

The details of the upper part of the instrument are shown in Figure 2. There is nothing critical about the dimensions; actually, the distance from the axis of the hinge to the micrometer is about 5.5 inches (140 mm.).

The wood pieces on which the optical parts are mounted are drilled to take the microscope tube and the 0.375-inch (10-mm.) rod supporting the slit. A longitudinal saw cut is then made, so that transverse bolts can hold the wood tightly around the microscope tube and the rod. The micrometer can be made by cutting off the frame of a 25-cent micrometer caliper. (Almost any screw will do, but it is convenient to have the scale of the micrometer caliper). The prism can be held on with wax or adhesive tape. The slit assembly is mounted on a 0.375-inch (10-mm.) rod which is held in the wood piece as described above.

It is convenient to be able to rotate the slit in order to make it parallel to the image of the spectral line. Care should be taken to have the slit short enough so that light will not strike the walls of the tube before reaching the photocell. A small piece of opal glass is placed in front of the photocell in order to spread the light over its surface. The photocell, opal glass, and slit are held together with rubber bands. A hood of black paper may be placed over the slit and prism in order to keep stray light off the slit.

The light source for reading galvanometer deflections is a Mazda No. 63, 3-candlepower, 6- to 8-volt automobile light, mounted horizontally just below a transparent celluloid scale. The scale is clamped between two pieces of wood cut to the proper curvature to keep all parts of the scale at the same distance from the galvanometer. The lamp and scale are so placed that an image of the filament of the lamp is formed on the scale.

No attempt has been made to choose the most suitable equipment. However, the parts listed below are satisfactory.

Microscope, Bausch & Lomb, Model H, No. 31-21-50-06 (32-mm. objective). Mechanical stage, Bausch & Lomb, No. 31-59-59. Voltage-regulating transformer, Raytheon, No. VR 107-A; output volts 6 to 7.5. Prism, Bausch & Lomb, No. 31-90-01-016. Slit, Gaertner, No. M710 (a slit from a spectrometer can be used). Photocell, General Electric, No. 4,120,833 Gl. Galvanometer, Leeds & Northrup, Type R, No. 2500-f. Sensitivity 10^{-10} ampere per mm. Galvanometer scale, Gaertner, No. M675.

Operation

In measuring a spectrogram, the photographic plate is clipped on the mechanical stage and focused on the slit. The image of a spectral line is brought near to the slit by adjusting the mechanical stage and the slit is turned until it is parallel to the image of the line. The image of the line is now centered on the slit roughly by adjusting the mechanical stage and precisely by turning the micrometer screw until the galvanometer deflection is a minimum. The density of the line is computed from this galvanometer deflection and that through the clear plate.

Performance

The magnitude of the random fluctuations in the galvanometer deflection was determined by taking 25 readings, one each 10 seconds. This was done several times and it was found that, with a deflection of from 45 to 50 cm., the average deviation of an individual reading from the mean never exceeded 0.1 cm., approximately 0.2 per cent of the reading. These fluctuations were probably caused by slight voltage fluctuations which the voltage regulating transformer failed to eliminate.

Since the light reaching the photocell strikes different portions of its surface as the micrometer is turned from one position to another, it is necessary to see that this causes no error. Tests show that no appreciable error is introduced by turning the micrometer a half turn either way from its normal position. In use, it need never be turned more than this.

A comparison of the calibration of a neutral-tint wedge made using this instrument with that furnished by the maker shows that the deflections are proportional to the light reaching the photocell for deflections from 4 to 40 cm., the range tested.

In order to show what precision may be expected in measuring actual spectral lines, the ratio of the deflections obtained with the instrument set alternately on a line of density 0.36 and on a line of density 0.60 was calculated. The average deviation of a single ratio from the mean of five was 0.5 per cent.

The time required to center the image of a spectral line on the slit and to read the galvanometer deflection is approximately one minute.

The instrument has been in use intermittently for over a year and, although it is rather slow, it has been found to yield entirely satisfactory results.

Silver Chloride Electrode in Acid-Base Titrations

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THE use of the silver–silver chloride electrode in potentiometric titrations has been suggested by Cavanagh (*1*, *8*) and by Hiltner (*6*). Cavanagh used the electrode in an ingenious scheme which obviated the necessity for a potentiometer in certain titrations, while Hiltner used the electrode, as well as the other silver–silver halide electrodes, in the titration of soluble halides. The general use of such an electrode in acid-base titrations, where it may replace the calomel electrode, does not seem to have been given the prominence it deserves. The advantages over the calomel electrode are that there is no possibility of plating mercury out on the hydrogen electrode; the liquid junction is avoided; and the experimental setup is simplified since the silver–silver chloride electrode is immersed directly in the acid being titrated. The characteristics of the silver–silver chloride electrode which make it unusually satisfactory are that it is a truly reversible electrode in the thermodynamic sense; it attains equilibrium almost instantaneously; and the developed potential is extremely stable.

The preparation of the silver chloride electrode has been described in detail numerous times by Harned and others. Briefly, it may be prepared by heating silver oxide on a platinum spiral at 450° C. and then electrolyzing in a hydrochloric acid solution (*4*, *9*), or by heating a mixture of 90 per cent silver oxide and 10 per cent silver chlorate on a platinum spiral at about 500° C. (*7*). While either type of electrode is satisfactory, the latter type is preferable, since it may be used for titrations over a period of months. Following a titration the electrode is washed carefully and kept in distilled water.

In titrating acids other than hydrochloric, such as sulfuric, the weak organic acids, etc., it is necessary to provide chloride ions or silver ions, this being done most conveniently by adding a small quantity of sodium chloride. The addition of a salt to the solution of a weak acid also has the virtue of causing a slight increase in the dissociation of the acid (*5*). For titrations of hydrobromic or hydriodic acid the corresponding halide electrode may be prepared according to the methods outlined above. The chief limit to the use of the electrode is that ions whose silver salts are very much less soluble than silver chloride should not be present.

During the titration the original concentration of chloride ions will be decreased, owing to the increase in volume, as the base is added. This change in chloride-ion concentration will alter the electrode potential of the silver chloride electrode somewhat, but in no case will the change of the hydrogen electrode potential at the end point be masked. The shape of the titration curve will thus be slightly different from that obtained with the calomel cell with liquid junction, but the position of the end point will not be shifted.

If the quinhydrone electrode is used in place of the hydrogen electrode, as may be done, the pH of the solution should not exceed 8.5 during the titration, because of the effect of more alkaline solutions on quinhydrone. This limitation does not apply to the silver chloride–hydrogen electrode combination.

The experimental procedure suggested here should not be used directly for the determination of pH without modification. The same pair of electrodes has been applied successfully to the exact determination of the activity and concentration of hydrogen ions by Hamer (*2*) and by Hamer and Acree (*3*). For titrations, however, both in instruction and in practical determinations, the electrode as described has been used in this laboratory for some years and may be recommended as a distinct improvement over the more usual calomel and salt bridge arrangement.

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A Time-Saving Adaptation for Photomicrography

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IN USING a chemical microscope to select a representative field, crossed nicols are often necessary. If a photomicrographic camera carrying a view tube is connected with the microscope, the camera must be removed for manipulation of a cap nicol analyzer and replaced to photograph the selected field; but if the photomicrograph is to be taken without the analyzer, with either polarized or unpolarized illumination, the adaptation depicted renders unnecessary the removal of the camera.

The analyzer support is clamped onto a slotted metal ring that fits over the view tube ocular. The analyzer and compensating devices can then be used without disturbing the camera.

Most view tubes are constructed with a reflecting prism in the light path above the ocular of the microscope. Because reflection reduces light vibrations parallel to the plane of incidence, the vibration direction of the analyzer should be adjusted perpendicular to the plane of incidence of the reflecting prism, so it can function in the same way as when crossed nicols are used with a microscope alone. This is easily effected by ascertaining the vibration directions of the analyzer and the substage polarizer and turning them to the proper positions relative to the prism.

The same result can be attained by using a microscope with a tube analyzer, but it is frequently impossible to obtain such an instrument, although the ordinary chemical microscope equipped with a cap nicol is fairly common.

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Organic Halogen Compounds in Mineral Oils

Detection, Determination, and Identification

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DURING the past fifteen years, and particularly during the past five years, addition of organic halogen compounds to various types of mineral oils has become a practice in the petroleum industry. These mixtures include a wide range of products, such as fireproof cleaning solvents, cutting oils, break-in oils, and special lubricants. In almost every case the halogen compound used in the mixture is a chlorine compound. Methods for the detection, determination, and identification of the organic halogen compound or compounds present in mineral oils are therefore necessary in the examination of such products.

Methods are included in the present paper for (1) the determination of total halogen (such as chlorine) in an oil, (2) the determination of the type of halogen compound present—i. e., whether the chlorine is or is not attached directly to an aromatic ring—and (3) the possible identification of the halogen compound present.

Determination of Total Halogen

A number of well-known methods have been advanced for the determination of halogen in organic compounds or in mineral oils, but these consume too much time to be used in ordinary laboratory operations. The following method, in which the hydrogen chloride formed during combustion of the mineral oil in a Parr oxygen bomb is absorbed by sodium bicarbonate solution (in the bomb), which is in turn titrated with standard silver nitrate solution by either the Mohr or Volhard procedure, is rapid and accurate and has given excellent results over a period of several years.

PROCEDURE. About 20 ml. of distilled water, in which is dissolved 0.500 gram of C. P. sodium bicarbonate, are placed in a Parr oxygen bomb of 400-ml. capacity. From 0.6 to 0.8 gram of the oil to be tested is placed in the weighed oil cup and the weight of the charge is determined to the nearest milligram. The cup is then placed in the bomb, which is previously arranged so that the iron firing wire (10 cm.) touches the oil sample when the cup is placed in position. The bomb is tightly closed and oxygen at 30 atmospheres is admitted, after which the ignition is carried out in the usual manner in a container of cold water. After ignition the bomb is allowed to cool (10 minutes) and the pressure is released at a uniform rate such that the operation requires not less than 1 minute.

The bomb is opened and the inside is examined for traces of unburned oil and sooty deposit. If either is found, the determination is discarded. If complete combustion has taken place, the interior of the bomb, including the oil cup, is rinsed with a fine jet of distilled water into a 400-ml. beaker. The washings usually do not exceed 350 ml. To the beaker is added 1 ml. of 2.5 per cent potassium chromate indicator solution and the halogen content is determined by titration with 0.05 *N* silver nitrate solution. In order to obtain a sharp end point, it is essential that the solution be viewed through bright yellow glass during the titration with silver nitrate solution. A blank determination must be carried out, in which 0.500 gram of the C. P. sodium bicarbonate, dissolved in exactly the same volume of water used in the beaker during the test, is titrated under the same conditions as when the sample was used. The halogen content is calculated in the usual manner after the quantity of silver nitrate solution used in the blank is subtracted from that used for the sample.

If the particular halogen present—bromine, chlorine, or iodine—is unknown, a second sample is burned in the bomb and the contents are examined by the usual qualitative procedure.

Typical results by the above procedure are shown in Table I.

TABLE I. DETERMINATION OF TOTAL HALOGEN IN LUBRICATING OIL

Halogen Compound Added	Halogen Calculated	Halogen Found
	%	%
n-Heptyl bromide	0.045	0.044
	0.450	0.466
n-Amyl chloride	0.333	0.324
Triphenylmethyl chloride	0.014	0.014
	0.142	0.140
Ethylene dichloride	0.069	0.067
	0.690	0.695
Benzyl chloride	0.028	0.029
	0.280	0.294
o-Dichlorobenzene	0.479	0.470
	0.048	0.050

Determination of Type of Halogen Compound Present

Procedures given in the literature and well-known qualitative organic texts are very indefinite with respect to experimental details for the determination of the type of halogen compound present. Potassium and sodium hydroxide solutions, both aqueous and alcoholic, have long been suggested as reagents for this purpose. It has been found that 1 *N* alcoholic potassium hydroxide solution when boiled 5 minutes with the sample does not react with halogen attached to an aromatic ring except in the case of iodine where a positive group such as a carboxyl or aldehyde group is in the ortho position. Practically all other organic halogen compounds are attacked by the alcoholic potassium hydroxide solution.

PROCEDURE. From 0.1 to 0.2 gram of the halogen compound or 8 cc. of mineral oil containing a halogen compound is placed in a 15-cm. (6-inch) test tube with 8 cc. of 1 *N* alcoholic potassium hydroxide solution (prepared from special potassium hydroxide containing not more than 0.002 per cent chlorine and 95

TABLE II. REACTIVITY OF ORGANIC HALOGEN COMPOUNDS

(With 1 *N* alcoholic potassium hydroxide and saturated silver nitrate solutions)

Positive Reaction with 1 *N* Alcoholic KOH		
Positive reaction with alcoholic $AgNO_3$	Negative reaction with alcoholic $AgNO_3$	Negative Reaction with 1 *N* Alcoholic KOH
Isopropyl bromide	Acetylene tetrachloride	Chlorobenzene
n-Butyl chloride	Trichloroethylene	Trichlorobenzene
Isobutyl bromide	Tetrachloroethane	*o*-Dichlorobenzene
n-Butyl bromide	Pentachloroethane	α-Chloronaphthalene
n-Amyl chloride	Tetrachloroethylene	*p*-Chlorodiphenyl
n-Amyl bromide		*p*-Bromoaniline
Isoamyl bromide		Chlorohydroquinone
Isoamyl chloride		Bromobenzene
n-Heptyl bromide		α-Bromonaphthalene
Triphenyl methyl chloride		Tetrachloronaphthalene
Benzyl chloride		*p*-Dibromobenzene
Benzyl bromide		*p*-Dichlorobenzene
Hexachloroethane		Iodobenzene
p-Phenylphenacyl bromide		
Chloroacetal		
Dichloropentane		
Chlorocyclohexane		
p-Nitrobenzyl bromide		
Butadiene tetrabromide		
Trichloroacetic acid		
Carbon tetrachloride		
Iodoform		
Ethylene dibromide		
Ethylidene bromide		
o-Iodobenzoic acid		
Slightly positive reaction with alcoholic $AgNO_3$		
Propylene dichloride		
Chlorex		
β-Phenoxy-β'-chloroethyl ether		
Monochloroacetic acid		
Methylene chloride		
Chloroform		
Ethylene dichloride		
1,1,2-Tribromoethane		

per cent ethyl alcohol). The test tube is placed in a steam bath for exactly 5 minutes; then 5 cc. of distilled water are added and the contents are filtered through Whatman No. 42 filter paper. The filtrate is acidified with dilute nitric acid; if turbid it is filtered through sufficient filter paper to give a clear solution. To this solution is added 0.5 cc. of 0.05 *N* silver nitrate solution and it is examined for turbidity due to silver halide. Blank tests on the reagents are carried out in the same manner. Any excess of turbidity over that given by a blank test on the reagents used indicates the presence of organic halogen not attached to an aromatic ring (except with a few iodine compounds as stated above).

The results obtained with a number of organic halogen compounds are shown in Table II. For purposes of comparison, results obtained by heating the halogen compound with a saturated solution of silver nitrate in absolute alcohol are also included.

The sensitivity of the test for the determination of organic halogen compounds in mineral oil is shown in Table III.

In case the two types of organic halogen compounds are present in the same sample of mineral oil, the halogen not attached to an aromatic ring may usually be removed from the oil by continued refluxing with 1 *N* alcoholic potassium hydroxide until the halogen content of the mineral oil decreases to a constant value, this latter value indicating the amount of halogen attached directly to an aromatic ring.

TABLE III. SENSITIVITY OF TEST FOR HALOGEN IN LUBRICATING OIL

(Where halogen is not attached to an aromatic ring)

Compound Present	Minimum Halogen Detected by Test %	Compound Present	Minimum Halogen Detected by Test %
n-Amyl chloride	0.002	Hexachloroethane	0.005
n-Heptyl bromide	0.002	Trichloroethylene	0.004
Benzyl chloride	0.001	Pentachloroethane	0.004
Chloroform	0.005	Ethylene dichloride	0.004
Carbon tetrachloride	0.005	Monochloroacetic acid	0.002
Triphenylmethyl chloride	0.001	Trichloroacetic acid	0.003
		Chlorex	0.003

Identification of Halogen Compound Present

Mineral oils containing halogen compounds may be distilled *in vacuo*, the halogen compounds usually being concentrated in one or more of the fractions obtained. Upon examining the results obtained by the methods mentioned above and the physical constants of the fractions, the halogen compound present usually is readily identified and a derivative may be prepared if desired. This examination of the fractions is time-consuming and may be replaced in many instances by a simple procedure by which the halogen compound may be extracted from the mineral oil as a thiuronium salt and identified by means of the corresponding thiuronium picrate.

PROCEDURE. One hundred grams of mineral oil and 50 cc. of C. P. benzene are refluxed for 2 hours with 10 cc. of 95 per cent ethyl alcohol containing 1 gram of thiourea. The alcohol layer is separated and the oil extracted with 25 cc. of hot 95 per cent alcohol, after which the two alcohol solutions are combined and evaporated to dryness on a steam bath. The resulting thiuronium salt is washed with cold hexane to remove traces of oil, then dissolved in 10 cc. of 95 per cent alcohol, and 1 gram of picric acid is added. The mixture is heated on the steam bath until complete solution is attained, then allowed to cool slowly. The thiuronium picrate is filtered off, washed with 5 cc. of cold 95 per cent alcohol, then repeatedly recrystallized from 95 per cent alcohol until the melting point becomes constant. Usually two recrystallizations are sufficient.

Table IV shows the melting points of the thiuronium picrates obtained by this procedure from several portions of lubricating oil to which had been added the compound listed.

TABLE IV. MELTING POINTS OF THIURONIUM PICRATES DERIVED FROM HALOGEN COMPOUNDS IN LUBRICATING OIL

Halogen Compound Added to Lubricating Oil (1% in Each Case)	Melting Point of Thiuronium Picrate Found (picrate derived from halogen compound in oil) ° C.	Literature value (*1*) ° C.
n-Amyl chloride	151	154
n-Heptyl bromide	138	142
Triphenylmethyl chloride	170	...
Ethylene dichloride	259	260
Benzyl chloride	188	188

Since no value for the melting point of *s*-triphenylmethyl thiuronium picrate was available in the literature, this compound was prepared by refluxing triphenylmethylchloride with thiourea in alcoholic solution, followed by addition of picric acid and recrystallization from alcohol. The melting point was 172° C. and analysis of the compound indicated 5.5 per cent of sulfur (theoretical, 5.85 per cent).

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PRESENTED before the Division of Petroleum Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Note on Determination of Silica in Calcined Alumina

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THE determination of traces of silica in calcined alumina required for the manufacture of aluminum presents a number of difficulties. The author has found that silica may be determined in calcined alumina colorimetrically as silicon molybdate in acid solution and that this method is quicker and more accurate than the gravimetric method. It may also be adapted to many estimations of small amounts of silica or silicon.

A Rapid Method for Determining Ferric and Ferrous Iron

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Using precipitated copper as a reducing agent, determinations of ferrous and ferric iron are carried out in less than 10 minutes, with a minimum of equipment and skill, with common stable reagents, in the presence of large amounts of cupric, ferrous, manganous, zinc, nickel, and chromic ions, and with a precision and accuracy of around 3 parts per thousand.

WITH many mills turning to the use of ferric sulfate for pickling brass and other copper alloys, the need for a simple rapid method for the determination of ferric ion in the baths has arisen. (Metals are pickled after high-temperature annealing to remove the oxide film and to produce a bright uniform appearance.)

Standard methods are unsatisfactory for this purpose because mills demand a method so simple and rapid that it can be carried out beside the bath by regular workmen without special training and without taking much time from their other duties. Thus, all methods are eliminated which take more than 10 minutes, which require laboratory skill, special apparatus, and unusual or unstable reagents, and which are expensive or inaccurate in the presence of large amounts of cupric ion.

The method developed to meet these requirements comprises reduction by finely divided copper, removal of the excess, and titration with standard permanganate.

Metallic copper has been used before for reducing ferric salts (*1*, *3–5*, *7–9*), but in all cases copper with a relatively small surface area per unit of weight, such as gauze, turnings, or foil, has been used. This makes the procedures several times longer than the present one and necessitates reduction in a boiling solution, which is an undesirable complication in itself. For example, Hendel (*5*), who made a study of the time required to reduce various amounts of iron, found 30 minutes' boiling with 32 sq. cm. of copper gauze was required to reduce 125 mg. of iron. This quantity, and more, is reduced in 2 minutes at room temperature by the method described.

No one has previously pointed out that this method is capable of determining the ferric-ferrous ion ratio in the presence of large amounts of cupric ion.

Materials

A glass-stoppered 250-cc. flask is preferable. Filtration facilities are desirable but not essential.

Potassium permanganate, 0.1 *N*. Commercial anhydrous ferric sulfate (Ferrisul).

Copper metal precipitated powder (a reagent electrolytic product was purchased, 98 per cent of which passed through a 325-mesh certified Tyler sieve and 84 per cent of which is in the range 10 to 35 microns as determined by a Wagner turbidimeter).

Other reagents used in tests were of ordinary laboratory C. P. grade.

Method

Add 50 cc. of water, 15 cc. of 20 per cent sulfuric acid, and a 4-cc. sample of filtered pickle bath to the 250-cc. flask. Titrate with 0.1 *N* potassium permanganate, add a slight excess of copper metal precipitated powder (0.1 to 0.2 gram), shake violently 2 minutes, filter, and titrate again. One half the net titer gives per cent of ferric sulfate present. For convenience in titration the size of sample may be adjusted to give a titer of 15 to 25 cc. There must be an excess of copper present at the end of the shaking, but an extremely large excess is not recommended in accurate work. For cases where an error of 1 per cent is not serious, the filtration may be eliminated by shaking 30 seconds with 2 cc. of mercury and titrating rapidly.

Calculations.

1 cc. of 0.1 *N* $KMnO_4$ = 0.019993 gram of $Fe_2(SO_4)_3$
= 0.02 gram of $Fe_2(SO_4)_3$ with error of only 0.035%
= 0.01519 gram of $FeSO_4$
= 0.0152 gram of $FeSO_4$ with error of only 0.066%

% salt = grams of salt in 100 cc. of solution

Cc. of $KMnO_4$ for 4 cc. of sample × 25 = cc. of $KMnO_4$ for 100 cc. of solution

Net cc. of 0.1 *N* $KMnO_4$ × 25 × 0.02 = cc. of 0.1 *N* $KMnO_4$ × 0.5 = % of $Fe_2(SO_4)_3$

(Cc. of 0.1 *N* $KMnO_4$, 1st titer, − Blank) × 25 × 0.0152 = cc. of 0.1 *N* $KMnO_4$ × 0.38 = % of $FeSO_4$

The three different Ferrisul solutions used in testing the method are designated as I, II, and III. Elaborate precautions or unusual care are not required and were not taken in this work except where necessary in the two standard procedures used. Duplicate analyses were made in all cases. The greatest deviation of the duplicate analyses from their average was ±0.5 per cent. The average deviation was 0.3 per cent. (These are percentages of actual value found; in terms of per cent ferric sulfate they are, for solutions I and II, 0.03 and 0.04 per cent, respectively.) Since this precision is entirely satisfactory for the purpose at hand, no attempt was made to determine the limits of precision attainable by this method. All per cent concentrations given are equivalent to grams of salt per 100 cc. of solution at room temperature.

Table I. Comparison with Standard Methods

	$Fe_2(SO_4)_3$ Found	
	Solution I	Solution II
	%	%
SO_2 method	8.73	..
Cu method	8.72	12.74
$SnCl_2$ method	..	12.71

Comparison with Standard Methods (Table I). The sulfur dioxide reduction was carried out according to a standardized procedure used here for plant control work.

Add the sample containing about 0.75 gram of ferric sulfate to 200 cc. of distilled water in a 500-cc. flask; raise the pH to point of precipitation and pass in a rapid stream of sulfur dioxide for 15 seconds. Bring to boil over 5-minute period (must be colorless) and add 10 cc. of 1 to 1 sulfuric acid. Boil rapidly until there is no odor of sulfur dioxide, or at least 10 minutes. Cool and titrate with 0.1 *N* potassium permanganate. Make blank correction. About 30 minutes are required.

The stannous chloride reduction and titration using the Zimmerman-Reinhardt preventive solution followed the directions of Swift (*10*). The agreement of the present method within about 2 parts per thousand with these standard methods is thus less than experimental error, and shows that the new method is accurate.

Effect of Other Ions. Table II shows that large amounts of ferrous, cupric, manganous, zinc, nickel, and chromic ions

Table II. Effect of Other Ions

(Ferrisul Solution III)

Added Salt	%	$Fe_2(SO_4)_3$ Found %
.....	..	8.83
$FeSO_4.7H_2O$	25	8.86
$CuSO_4.5H_2O$	25	8.81
$MnSO_4.2H_2O$	62	8.78
$ZnSO_4.7H_2O$	100	8.87
$NiSO_4.6H_2O$	42.5	8.87
$Cr_2(SO_4)_3$	3	8.81
NaCl	25	13.1

do not affect the method outside of experimental error. Chloride, as would be expected, caused interference.

Discussion

Aside from the simplicity of a copper reduction method, it has the all-important advantage here of being applicable directly to brass pickling baths containing large amounts of cupric ions, which would not be the case with a zinc or stannous chloride reduction. The fact that chloride is not required as in a mercury (*2*, *6*) or silver (*11*) reduction makes it possible to use a simple permanganate titration. The absence of special apparatus, such as a reductor tube or colorimetric standards, makes the method particularly suited to millwork where such equipment could be subjected to careless handling and breakage. The simple equipment used in this method is easily replaced without the attention of a trained chemist.

The method requires no special skill and the accuracy and precision of the results show that interference by cuprous compounds and air oxidation is negligible. The use of precipitated copper powder so accelerates the reduction that the method becomes one of the fastest and simplest of analytical methods, which should recommend its use in other applications. The precision of the results is consistent with the technique used and no results were obtained which would indicate that the method could not be made even more precise than 3 parts per thousand, if desired, by introducing refinements.

Acknowledgment

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Volumetric Determination of Iron and Aluminum in Cement with 8-Hydroxyquinoline

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In the usual procedure (*2–10*, *11*, *14*) for the determination of iron and aluminum with 8-hydroxyquinoline, the acidity is generally set with acetic acid and ammonium acetate at a pH close to 7, and an acetic acid solution of the 8-hydroxyquinoline is added to precipitate the metals.

Results obtained by this procedure are erratic. While the gravimetric results are satisfactory, the volumetric results are consistently low; although the metals are completely precipitated, they do not come down completely as the oxine. This is due to the fact that the pH for precipitation as the oxine is the same as for precipitation of these elements as the basic acetates. Moyer and Remington (*12*) have shown that iron is precipitated completely with oxine at a pH of 3.45 to 4.00 and aluminum at a pH of 5.0. At both these points the basic acetate precipitates. When the metals are ignited to the oxides the results are satisfactory, but when the oxine precipitate is weighed, the results are a little low because the basic acetates are lighter than the oxines. In the volumetric determination the metal which comes down as the basic acetate is not determined. The amount of error which this procedure can cause will depend on the dilution, the temperature, and the time of heating, all of which favor the formation of the basic acetate.

To precipitate both iron and aluminum, the pH must be above 5.0. The addition of tartaric acid prevents the formation of the basic acetate at this pH, and the metals can then be precipitated completely as the oxine.

Since divalent iron reacts with only 2 instead of 3 moles of the oxine, it is necessary to boil the solution with bromine water before adding the oxine.

Two methods for the titration of the oxine precipitate are used. The first—to titrate the oxine directly with a bromate solution before using neocarmine, methyl red, or other dye as the indicator—is not generally used because the color of the dye fades gradually and the end point is not sharp (*7*, *13*). The second method—to add an excess of the bromate and back-titrate with potassium iodide and thiosulfate—cannot be used in the titration of the iron oxine because the potassium iodide also reacts with the ferric ion.

The poor end point with the first method was therefore studied. It was found that the color produced in this titration was not reversible. A very small amount of the bromate solution is able to decolorize a large amount of the indicator. Thus, during the titration, the indicator is being used up by the bromate. To eliminate this it is only necessary to add more indicator when the solution starts to become decolorized. Another reason for the poor end point is that when methyl red, for example, is used in a weak acid solution, the color change is from red to orange to green to yellow. The final transition from green to yellow is very indistinct and it is easy to overrun the end point by as much as 1 cc. If, however, the acid concentration is above 15 per cent, the green color is eliminated and a sharp transition from orange to yellow will be observed, the red changing to orange just before the end point is reached. A satisfactory end point can then be obtained by direct titration with the bromate solution.

Results obtained with these modifications as shown in Table I are typical of many similar determinations.

To determine the iron and aluminum volumetrically the two metals can be precipitated together as the oxine and titrated as indicated above. The iron can then be determined volumetrically on a separate portion in the usual way, or determined on the same portion oxidimetrically.

To determine iron and aluminum in the same solution, both were precipitated with oxine and titrated with the bromate solution; then the iron was titrated oxidimetrically in the same solution. The first titration gave satisfactory reproducible results, but the second did not. When the iron was titrated with potassium permanganate by the Zimmerman-Reinhardt method, the very high results were due, apparently, to an oxidation of the oxine. An oxidizing agent with lower potential (potassium dichromate) gave better results but still high. The iodometric method, which has a much lower potential, was then tried and, after modification, gave satisfactory results as shown in Table I.

Table I. Determination of Iron and Aluminum

Sample	Metal Present		Bromate Titration	Thiosulfate Titration	Error
		Gram			*Mg.*
1	Al_2O_3	0.0107	0.0108	....	+0.1
2	Al_2O_3	0.0270	0.0267	....	−0.3
3	Al_2O_3	0.0480	0.0480	....	0.0
4	Fe_2O_3	0.0080	0.0081	....	+0.1
5	Fe_2O_3	0.0186	0.0185	....	−0.1
				0.0187	+0.1
6	Fe_2O_3	0.0362	0.0363	....	+0.1
				0.0361	−0.1
7	Al_2O_3	0.0261	0.0267	....	+0.6
	Fe_2O_3	0.0196	....	0.0195	−0.1
8	Al_2O_3	0.0262	0.0259	....	−0.3
	Fe_2O_3	0.0170	....	0.0173	+0.3
9	Al_2O_3	0.0346	0.0341	....	−0.5
	Fe_2O_3	0.0116	....	0.0114	−0.2

Procedure

Dissolve 0.5 gram of cement in perchloric or hydrochloric acid, depending on whether the silica is determined by the perchloric acid or the ammonium chloride method. After removal of the silica, add an excess of 10 ml. of hydrochloric acid, 2 drops of methyl red, and 5 ml. of 20 per cent tartaric acid. Make just alkaline with concentrated ammonium hydroxide and add 1 to 10 hydrochloric acid till a slight red color appears. Add 20 ml. of a 25 per cent solution of ammonium acetate and bring the solution to a boil. This procedure sets the pH at 5.8; however, it drops to 5.0 after precipitation, because of the release of hydrogen ions in the formation of the oxine complex and because the reagent contains acetic acid.

Cool slightly and add an excess of the 8-hydroxyquinoline reagent (shown by the yellow color of the supernatant liquid). Prepare the reagent by dissolving 12.5 grams of oxine in 30 ml. of glacial acetic acid and diluting with water to 1 liter. Let the precipitate stand 15 to 30 minutes, filter through a coarse filter paper, such as Whatman 41H, keeping the precipitate at least 1.25 cm. (0.5 inch) from the top of the paper because the iron oxine creeps excessively. Wash with warm water. Wash the precipitate back into the original beaker with a stream of hot water and pour 50 ml. of hot 1 to 2 hydrochloric acid over the filter paper to dissolve the remaining precipitate into the original beaker. Heat carefully till the precipitates just dissolve. Cool, add 2 drops of methyl red, and titrate with 0.2 *N* bromide-bromate solution. When nearing the apparent end point, add another 2 drops of methyl red, allowing 15 seconds before continuing the titration. Continue adding 2 drops of the indicator and titrating until the color changes to an orange and then a yellow within 15 seconds after adding the indicator. For greater accuracy, correct for the amount of bromate used up by the indicator by counting the number of drops of indicator added and determining on a blank the bromate equivalent. Fifteen drops of 0.5 per cent methyl red are decolorized by 0.10 ml. of bromate solution and 10 to 15 drops of indicator are generally used up in a titration.

Add a few drops of the oxine and cool to 15° to 20° C. Add about 6 grams of sodium carbonate and then 20 ml. of 15 per cent potassium iodide. After 5 minutes add 10 cc. of concentrated hydrochloric acid and titrate with 0.02 *N* thiosulfate, adding starch when the solution becomes a light yellow. Any color that develops after this titration should be ignored (this is due to the decomposition of the potassium iodide which occurs at the high acidity necessary to keep the iron oxine in solution). The iodometric titration must be run immediately after the bromometric titration because the iron is reduced on standing.

Table II. Interference

Sample	Al_2O_3 Present	P_2O_5 Present	Al_2O_3 Found
	Gram	*Mg.*	*Gram*
10	0.0267	5	0.0264
11	0.0267	10	0.0261
12	0.0267	15	0.0257
13	0.0267	20	0.0255
14	0.0267	25	0.0257

The bromate solution can be standardized with potassium iodide and thiosulfate. The thiosulfate can be standardized against potassium dichromate, or both solutions can be standardized by using the above procedure with Bureau of Standards' Sibley iron ore 27b or standard iron wire.

The interfering elements are large amounts of silica (which causes slightly lower results), copper (*4*), cadmium, cobalt, nickel, zinc, titanium, and zirconium. Less than 5 mg. of phosphate do not interfere. Table II shows the interference when more than 5 mg. are present. Magnesium and calcium do not interfere. The only interfering elements in this list, besides silica, that are likely to occur in cements are titanium and zirconium.

This method has the following advantages for routine laboratory analysis over the ammonium hydroxide method. It is free from most of the errors indicated by Hillebrand and Lundell (*7*): phosphate ion interference, traces of silica in the sample and in the reagents (especially ammonia), improper ignition (aluminum oxide requires 1200° C.), absorption of moisture by the ignited oxides, etc.

After the iron and aluminum are determined as above, the calcium can be precipitated at the same pH by the addition of ammonium oxalate and also determined volumetrically. Magnesium can then be precipitated by heating to 70° C., adding more oxine, then 30 ml. of concentrated ammonium hydroxide, stirring for 15 minutes, and letting settle 10 minutes. It is determined volumetrically in the same manner as the iron and aluminum. Cements contain up to 1 per cent of P_2O_5. This amount does not interfere with the oxine method but is carried down in the ammonium hydroxide method. Consequently, this method, in the presence of this ion, will give lower results than the A. S. T. M. method (*1*). Samples 7, 8, and 9 are cements with only a trace of P_2O_5.

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Spectrophotometric and Biological Assay of Vitamin A in Oils

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THE discovery in 1928 by Morton and Heilbron (*15*) that vitamin A has a characteristic absorption band in the region 320 to 330 $\mu\mu$ has been followed by numerous publications on the use of this physical fact as a means of quantitative measurement of the amount of vitamin A in oils. Such literature covers the improvement in experimental technique, irrelevant absorption, proper solvents, forms of vitamin A, and, dependent on all these, the correlation of results obtained by physical tests with biological assay through the proper conversion factor.

There is now available a wide variety of spectrophotometers and photoelectric photometers used in the physical assay of vitamin A. The Vitamin Assay Committee of the American Drug Manufacturers' Association (*1*) in 1937 and again in 1939 made an attempt to correlate results obtained by such instruments. An examination of these reports reveals a divergence among the values of extinction coefficients obtained by different instruments on the same oils, attributed partially to errors in the instruments and partially to the technique of the operator and experimental conditions.

The question of irrelevant absorption, or the presence in oils of substances which show ultraviolet absorption, not necessarily selective, in the region of 328 $\mu\mu$ is of great importance in the physical assay of vitamin A. Attempts to correct for such absorption have involved the application of correction factors (*3*, *12*, *16*) or the suggestion of Hume and Chick (*9*), who state that the irrelevant absorption bears no constant relationship to the amount of vitamin A present, that it is less for the unsaponifiable fraction than for the oil itself, but that in oils with large concentrations of vitamin A the error is insignificant. On the other hand, the problem is further complicated by the question as to whether the ultraviolet absorption at 328 $\mu\mu$ is a measurement of all the substances which cause vitamin A activity in rats.

There are two aspects to this problem. In the first place Gillam *et al.* (*5*) and Edisbury *et al.* (*4*) report the presence in oils of a new vitamin, vitamin A_2, which also has biological activity, but with ultraviolet absorption maximum at 345 to 350 $\mu\mu$. One other vitamin in whale liver oil with absorption maximum at 290 $\mu\mu$ has been reported by Willstaedt and Jensen (*17*), and it is possible that other forms of the vitamin exist. In the second place, Gray, Hickman, and Brown (*6*) report that vitamin A in fish liver oils is present mainly in the ester form. It is further implied by Hickman (*7*) that the esters of vitamin A have a greater biological activity than the alcohol. This may be due to the fact that the ester is more stable than the alcohol, as Mead, Underhill, and Coward (*13*) have found.

All these factors influence the value of the conversion factor. The normal procedure of obtaining the conversion factor is by direct computation from the E and biological values of the pure substance. In the case of vitamin A this procedure is not logical because of the instability of the vitamin and because some substances which have biological activity similar to vitamin A do not have absorption maxima at 328 $\mu\mu$. The value of 1600 first reported (*10*) was based not on pure vitamin A but on the richest concentrate known at the time. Since that time many values have been reported.

Hume and Chick (*9*) state that the values vary from 1300 to 2580. Morgan *et al.* (*14*) indicate that the factor may not be the same for cod liver oils as for oils of higher potency. Holmes and Corbett (*8*) report on tests made on crystalline vitamin A values from 1600 to 1800; one test by Darby gave a value of 2100. The 1939 report of the A. D. M. A. committee gave conversion factors ranging from 1920 to 2780. Barthen and Leonard (*2*) on tests on U. S. P. standard oil report a value of 2222; Mead, Underhill, and Coward (*13*) report values of 1920 and 2150 for two esters and, having made the correction factor for the acid fraction of the ester molecules, compute 2000 as the conversion factor for vitamin A. Hickman (*7*), using data obtained mainly from molecular distillation studies, states that the conversion factor for the esters of vitamin A is higher than for the alcohol, that each preparation has a characteristic conversion factor, and that cod liver oils in general have higher factors than most other fish liver oils.

It would thus seem that, until some of these matters are further clarified, each laboratory should obtain its own conversion factors by direct comparison between the E and biological values of a great variety of oils. Such a procedure has been adopted in this laboratory.

Experimental Procedure

PHYSICAL. Two instruments, the Hilger vitameter and the ultraviolet spectrophotometer, were used in the physical assay of the oils. Experience with the vitameter has shown that variations in its performance lead to rather inaccurate results. The vitameter, however, is a simple and speedy instrument to operate and has been found excellent for measurements preliminary to the biological and more accurate physical assays.

The spectrophotometer used was a Judd Lewis ultraviolet photometer with vanes carefully refinished and reset and the various units rigidly mounted to the frame of a 10 × 25 cm. (4 × 10 inch) Bausch and Lomb ultraviolet spectrograph. The original mounting of the Judd Lewis photometer has been re-

TABLE I. U. S. P. REFERENCE COD LIVER OIL

Oil No.	$E^{1\%}_{1\text{ cm.}}$	$\Delta E^{1\%}_{1\text{ cm.}}$[a]	Conversion Factor[b]
	Full Bottles		
1	1.40 (unsap.)	..	2140
2	1.36 (unsap.) 1.49 (whole)	0.13	2210 2010
3	1.39 (unsap.) 1.49 (whole)	0.10	2160 2010
4	1.36 (unsap.) 1.49 (whole)	0.13	2210 2010
	Partially Filled Bottles[c]		
5	1.24 (unsap.)	..	2420
6	1.12 (unsap.)	..	2680
7	1.15 (unsap.)	..	2610
8	1.17 (unsap.)	..	2560
9	1.17 (unsap.) 1.26 (whole)	0.09	2560 2390
10	1.14 (unsap.) 1.26 (whole)	0.12	2630 2390
11	1.24 (unsap.) 1.32 (whole)	0.08	2420 2270

Average conversion factor for unsaponified fraction
Full bottles 2180 ± 1.4%
Partially filled bottles 2550 ± 3%

Average conversion factor for whole oil
Full bottles 2010 ± 0%
Partially filled bottles 2350 ± 2%

[a] $E^{1\%}_{1\text{ cm.}}$ whole − $E^{1\%}_{1\text{ cm.}}$ unsap.
[b] Biological value taken as 3000 U. S. P.
[c] Flushed with carbon dioxide and allowed to stand in refrigerator for several weeks.

TABLE II. COD LIVER OILS

Oil No.	$E^{1\%}_{1\text{ cm.}}$	$\Delta E^{1\%}_{1\text{ cm.}}$ [a]	U. S. P. XI (Biological)	Conversion Factor
			Units per gram	
1872 (unsap.)	0.75	0.25	2050	2730
(whole)	1.00			2050
1891 (unsap.)	1.10	0.26	3100	2820
(whole)	1.36			2280
1870 (unsap.)	0.76	0.07	2300	3030
(whole)	0.83			2770
1885 (unsap.)	0.87	0.08	2400	2760
(whole)	0.95			2530
1847 (unsap.)	0.75	0.10	2400	3200
(whole)	0.85			2820
1838 (unsap.)	0.63	0.25	1800	2860
(whole)	0.88			2050
1859 (unsap.)	0.84	..	2000	2380
1857 (unsap.)	0.66	0.18	2300	3490
(whole)	0.84			2740
1928 (unsap.)	0.64	0.06	1650	2580
(whole)	0.70			2360
1667 (unsap.)	0.66	0.06	1800	2730
(whole)	0.72			2500
1850 (unsap.)	2.33	0.42	6000	2570
(whole)	2.75			2190
1833 (unsap.)	0.77	0.08	2250	2920
(whole)	0.85			2650
1697 (unsap.)	0.87	0.04	2050	2360
(whole)	0.91			2250
1699 (unsap.)	0.82	0.09	2150	2620
(whole)	0.91			2360
1903 (unsap.)	0.70	0.09	1700	2430
(whole)	0.79			2150
1879 (unsap.)	0.80	..	2150	2690
1978 (unsap.)	0.84	0.10	2150	2560
(whole)	0.94			2290
1987 (unsap.)	0.68	0.18	1600	2350
(whole)	0.86			1860
2013 (unsap.)	0.57	0.09	1550	2720
(whole)	0.66			2350
2004 (unsap.)	0.55	0.03	1400	2550
(whole)	0.58			2410
2021 (unsap.)	0.78	0.02	2100	2700
(whole)	0.80			2620
2022 (unsap.)	0.82	0.11	2000	2440
(whole)	0.93			2150
Average conversion factor for unsaponifiable fraction				2700 ± 8%
Average conversion factor for whole oil				2370 ± 9%

[a] $E^{1\%}_{1\text{ cm.}}$ whole − $E^{1\%}_{1\text{ cm.}}$ unsap. One per cent refers to concentration of whole oil before saponification.

placed by a more rigid and better aligned single unit mounting designed by the Squibb staff. In this mounting all the photometer units, including the source, are attached to the same unit. With such a mounting it has been the experience in this laboratory that in the 8 months of use, once the new photometer setup had been brought into adjustment (using approximately thirty plates), the only subsequent adjustments have been incidental to the removal and sharpening of the electrodes as they have worn away with use. On replacement of the electrodes a single plate taken varying the height of the arc was sufficient to reset the source.

The light source used was a tungsten-steel spark. On each assay plate one exposure with both apertures fully open and no absorbing cells in position was made as an adjustment check on the equality in intensity of the two beams. The density scale of the lower sector of the photometer was calibrated by comparing values found with solutions of potassium chromate and potassium nitrate with the values recorded in literature. Such concentrations were used as would include the entire scale. All absorption maxima of these absorbing salts were used. Such calibration was checked on the average after every ten plates had been exposed.

Eastman spectroscopic plates No. II O were used. Exposures with such plates, using Bausch & Lomb 1-cm. cells and slit width of 0.03 mm., varied from 2 to 10 seconds. Plates were developed for 5 minutes (at 20° C.) in Eastman D19 developer, washed and fixed for 15 minutes, then washed in running water for at least 30 minutes.

The actual procedure in the assay of any oil was to weigh out directly into a 100-ml. flask a quantity of the fresh oil accurate to a 0.1 mg. This was then diluted with isopropanol and a vitameter reading was taken. Dilutions that would give a match at a density reading of between 0.50 and 0.95 were used. A similar concentration was then placed in one of the photometer cells with isopropanol in the compensating cell, and a plate was taken varying the aperture of the density scale from 0.15 to 0.95 by steps of 0.05. On such a plate it was possible to read the match point at 328 $\mu\mu$ to 0.05 density readings and also to plot the absorption curve of the oil. When such a density reading was obtained the value of $E^{1\%}_{1\text{ cm.}}$ was calculated from Beer's law and a second plate was taken. On this second plate two or three concentrations were used, each covering from six to nine exposures. The test solution was thus exposed to the ultraviolet light for not more than 1.5 minutes.

Tests made for deterioration of the oils due to irradiation from the source when the cells were in their normal position with respect to the source and the aperture was fully open indicated that for exposures up to 3 minutes the decrease in the E value was well within the error of the instrument. A second weighing was also made and a third plate taken with two or three concentrations. If the extinction coefficients computed from the plates agreed to within 5 per cent for the different weighings no further data were taken; if not, a third weighing was made and plate exposed.

Plates, when dry, were placed on a well-illuminated viewing stand and examined visually with a jeweler's loop for density match points at 328 $\mu\mu$. If there was doubt about any match point, the plate was projected on a screen using a Bausch & Lomb Balopticon projector and the projected image was examined for the match point.

With the lower potency oils assays were made on both the whole oil and the unsaponifiable fraction. Two methods of saponification were used—one, that suggested by the Vitamin Assay Committee of the A. D. M. A. of 1937 (*1*) with the additional procedure that the ether was evaporated under carbon dioxide, and the other a modified form of this procedure by which in the process of saponification and extraction of 1 gram of oil larger amounts of ether, alcohol, and water were used than in the first procedure.

Following this procedure, an accurate determination on a cod liver oil can be made in about 6 hours and on an oil of higher potency in 4 hours.

BIOLOGICAL. The U. S. P. XI procedure was followed in all cases. A master curve was used as an aid in interpreting the results and to increase the accuracy. Precautions were taken in the biological assay to avoid the use of U. S. P. reference oil which had been exposed to air or had been stored in partially filled bottles any appreciable length of time.

Fresh bottles of the oil, as distributed by the U. S. Pharmacopœia Vitamin Committee, were taken at intervals of 2 to 3 months, and subdivided into three or five small vials which were thoroughly flushed, sealed, and stored in a refrigerator. These small vials were then consumed in periods not exceeding 2 to 3 weeks. These vials and various diluted solutions which were used in the tests were always thoroughly flushed with carbon dioxide after being opened and then returned to the refrigerator. Fresh dilutions were prepared weekly.

Results

In Table I values obtained for the U. S. P. reference cod are tabulated. Each E value recorded represents an average value obtained by following the procedure outlined above for the assay of cod liver oils. In some cases more than two saponifications were made. In Tables II to IV the results for a series of oils are recorded. The conversion factors were obtained by dividing the biological value by the E value. The average conversion factors for the various types of oils and the average per cent errors were calculated.

Many absorption curves of the oils have been plotted. For the oils of higher potency the curves are fairly symmetrical about a pronounced peak around 328 $\mu\mu$. In some of these there is evidence of a "flat" in the curve at 310 and 340 to 350 $\mu\mu$. A comparison of the curves of the whole oils and unsaponifiable fractions of cods is interesting. In both cases there is a pronounced peak at approximately 328 $\mu\mu$, also evidence of a flat at 310 and 340 to 350 $\mu\mu$, but the curves for the whole oils are more irregular, showing evidence of selec-

Table III. Tuna and Halibut Liver Oils

No.	$E^{1\%}_{1\text{ cm.}}$	U. S. P. XI (Biological)	Conversion Factor
		Units per gram	
		Tuna Liver Oil	
1959	58	120,000	2070
1902	12	26,000	2170
1897	34	80,000	2350
1909	49	75,000	1530
1623	56	145,000	2590
1741	12	30,000	2500
1916	23	55,600	2420
1917	26	55,300	2130
2016	55	110,000	2000
2023	44	92,000	2090
Average conversion factor		2180 ± 10% 2260 ± 9% (omitting 1909)	
		Halibut Liver Oil	
1617	67	150,000	2240
1619	33	70,000	2120
1863	56	140,000	2500
1860	41	110,000	2680
1936	46	100,000	2180
1937	50	105,000	2100
1914	26	61,700	2380
1915	18	42,400	2360
1692	31	62,000	2000
1988	26	56,000	2150
1989	37	80,000	2160
1990	93	202,000	2170
Average conversion factor		2250 ± 6%	

tive absorption in the region 260 to 280 $\mu\mu$, and the curves as a whole are higher than those for the unsaponifiable fractions.

Discussion of Results

An examination of Table I reveals the fact that the E values listed for the U. S. P. reference cod liver oil vary over a considerable range from the higher consistent values for the assays on full bottles to the lower varied values on bottles which were only partially filled and were handled as indicated above. A similar falling off of the E values for this reference oil was reported by McFarlan (*11*).

There has been no evidence that any loss of vitamin A has occurred in the original bottles of U. S. P. reference samples as determined by repeated physical tests and biological assays. Whether or not the decrease in the E values which occur in the U. S. P. reference oil after standing for some time in partially filled bottles is also accompanied by a decrease in vitamin A activity has not been determined but is not believed to affect the interpretation of the biological assays, since the use of such oil has been avoided.

In Table II are listed the conversion factors for 22 cod liver oils as obtained by direct computation of the biological and physical values for the individual oils. The average of the unsaponifiable fraction for these oils is 2700 and that for the whole oils 2370. Conversion factors for oils of higher potency—tuna, halibut, and other fish liver oils—are listed in Tables III and IV, giving average values of 2260, 2250, and 2270, respectively.

It would appear that the conversion factors of the cod liver oils or at least a part of them were higher than that of the U. S. P. reference cod liver oil or samples of the more highly active tuna, halibut, and other fish oils which were studied. These differences cannot be explained by variations in the instrument, since the various oils have been run as received in the laboratories. Different oils were run concurrently and results should be comparable. Furthermore, the instrument was calibrated at frequent intervals with standard inorganic solutions. Neither is it considered that these differences are due to the loss of the vitamin in the process of saponification, since two methods of saponification were used and results agree to within 5 per cent. Moreover, the values for the whole oils themselves are greater than those for the other oils.

There is no explanation at the present time for the higher conversion factor, but the inference might be made that there are present in cod liver oils, in proportions greater than in oils of higher potency, substances which have biological activity similar to that of vitamin A but do not have maximum ultraviolet absorption at 328 $\mu\mu$. Further work on this point is in progress.

Summary

The details of a spectrophotometric method of assay of vitamin A in fish liver oils and the results of the biological and physical assay of 53 such oils are recorded. In all cases assays were made on fresh oils and all biological assays were made on the whole oils.

The average conversion factors computed from the measurements on 22 cod liver oils yield values of 2700 and 2370 for the unsaponifiable fractions and whole oils, respectively.

The average conversion factors for oils of higher potency are 2260 for tuna liver oils, 2250 for halibut liver oils, and 2270 for miscellaneous oils as listed, giving an average of 2260 for these oils of higher potency.

Studies on the U. S. P. reference standard have shown that the E value gradually decreases when the oil remains in partially filled bottles, even though they have been flushed with carbon dioxide and stored in a refrigerator.

Table IV. Miscellaneous Oils

No.	Type of Oil	$E^{1\%}_{1\text{ cm.}}$	U. S. P. XI (Biological)	Conversion Factor
			Units per gram	
1839	Pollack	3.8	10,500	2760
1878	Pollack	4.5	9,500	2110
1899	Pollack	3.7	8,500	2300
1948	Pollack	3.5	7,800	2230
1977	Pollack	4.2	10,200	2430
1934	Shark	56	135,000	2410
1944	Shark	57	109,000	1910
1979	Sword	28	67,000	2390
2019	Shark	109	206,000	1890
	Average conversion factor		2270 ± 9%	

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Polarographic Determination of Nickel and Cobalt

Simultaneous Determination in Presence of Iron, Copper, Chromium, and Manganese, and Determination of Small Amounts of Nickel in Cobalt Compounds

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THE purpose of this paper is to describe conditions under which nickel and cobalt can be determined simultaneously by the polarographic technique with the dropping mercury electrode (*2*). Heretofore an ammoniacal supporting electrolyte has been recommended for the simultaneus polarographic determination of nickel and cobalt (*5*, *8*, *9*). However, the degrec of separation of the two waves in ammoniacal medium is none too good, and accurate determination of the nickel is not possible when a large amount of cobalt is present (compare curve 2, Figure 1). The authors have found that an excellent separation of the nickel and cobalt waves is obtained in supporting electrolytes containing thiocyanate or pyridine. The use of these supporting electrolytes makes possible the rapid determination of small amounts of nickel present as an impurity in cobalt compounds, and greatly improves the simultaneous determination of nickel and cobalt in iron products (steel).

Apparatus and Experimental Technique

The usual polarographic technique was employed, using a Heyrovsky-Shikata type of polarograph for the automatic photographic recording of the polarograms (*1*, *2*). The H-type of cell, with a permanent external calomel electrode as anode, and the arrangement of the dropping electrode described by Lingane and Laitinen (*4*) were used for most of the measurements. Air was displaced from the cell solutions with nitrogen; the commercial tanked gas proved to be sufficiently pure for the present purpose. All measurements were made with the cell in a water thermostat at 25° C.

Comparison of Nickel-Cobalt Waves in Various Supporting Electrolytes

The polarogram in Figure 1 shows the waves obtained with a mixture of 0.001 *M* nickel chloride and 0.002 *M* cobalt chloride in different supporting electrolytes. Each solution contained 0.01 per cent gelatin as a maximum suppressor (*2*). The half-wave potentials of nickel and cobalt in these media are listed in Table I.

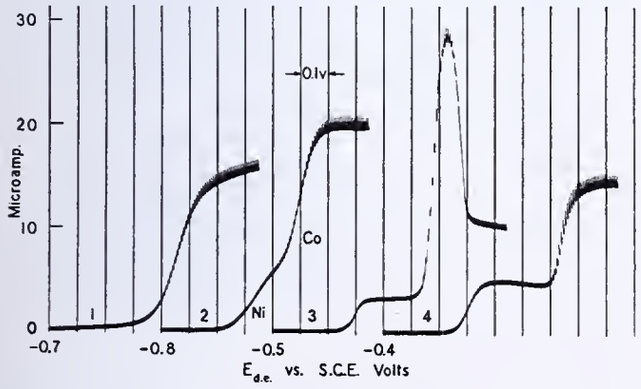

FIGURE 1. WAVES OF A MIXTURE OF NICKEL AND COBALT IN VARIOUS SUPPORTING ELECTROLYTES IN PRESENCE OF 0.01 PER CENT GELATIN

1. 0.001 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCl
2. 0.001 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* NH_4Cl-1 *N* NH_4OH
3. 0.0007 *M* $NiCl_2$ and 0.0013 *M* $CoCl_2$ in 1 *N* KCl containing 0.5 *M* pyridine
4. 0.001 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCNS

Starting potential of each curve indicated on abscissa, and distance between each vertical line corresponds to 0.1 volt

TABLE I. HALF-WAVE POTENTIALS OF NICKEL AND COBALT IN DIFFERENT SUPPORTING ELECTROLYTES

(Half-wave potentials in volts with respect to the saturated calomel electrode at 25° C.)

Supporting Electrolyte	$E_{1/2}$ Ni	$E_{1/2}$ Co	Difference
1 *N* KCl	−1.1	−1.2	0.1
1 *N* NH_4Cl-1 *N* NH_4OH	−1.12	−1.30	0.18
1 *N* KCl + 0.5 *M* pyridine	−0.78	−1.07	0.29
1 *N* KCNS	−0.70	−1.03	0.33

In a non-complex-forming supporting electrolyte the half-wave potentials of nickel and cobalt are so close together that a mixture of the two produces only a single wave, as shown by curve 1 in Figure 1. The coalescence of the waves in a non-complex-forming supporting electrolyte is further favored by the fact that the reduction of the aquo nickel and aquo cobaltous ions is irreversible, and the waves have an abnormally small slope.

In ammoniacal medium the difference between the half-wave potentials of the complex nickel and cobalt ammonio ions is large enough so that a double wave is obtained, as shown by curve 2 in Figure 1. However, the waves are still not sufficiently separate to allow an accurate measurement of their individual heights, especially if the concentrations of nickel and cobalt are disproportionate.

In pyridine or thiocyanate supporting electrolytes the half-wave potentials of both the nickel and the cobalt are shifted markedly to a more positive value but the nickel wave is shifted more than that of the cobalt; hence, in such solutions an excellent separation of the two waves is obtained, as shown by curves 3 and 4 in Figure 1. The complex ions formed in pyridine and thiocyanate solutions are reduced reversibly, as is evidenced by the normal slopes of the waves. Furthermore, the half-wave potentials in pyridine or thiocyanate solutions are about what one would expect from the ordinary standard potentials of nickel and cobalt combined with a reasonable estimate of the dissociation constants of the complex ions. This matter will be discussed in a later paper.

It will be noted from Figure 1 that the wave of cobalt in pyridine solution has a very prominent maximum, in spite of the presence of gelatin, but the diffusion current following the maximum is well defined. The authors attempted to suppress the cobalt maximum in 0.5 *M* pyridine by increasing the concentration of gelatin, with the results shown in Figure 2. The small nickel maximum is easily eliminated with only 0.01 per cent gelatin, but the cobalt maximum is not suppressed even with 0 05 per cent gelatin.

The cobalt maximum in pyridine solution in the presence of 0.05 per cent gelatin is not obtained when the concentration of pyridine is smaller than about 0.2 or 0.3 *M* (Figure

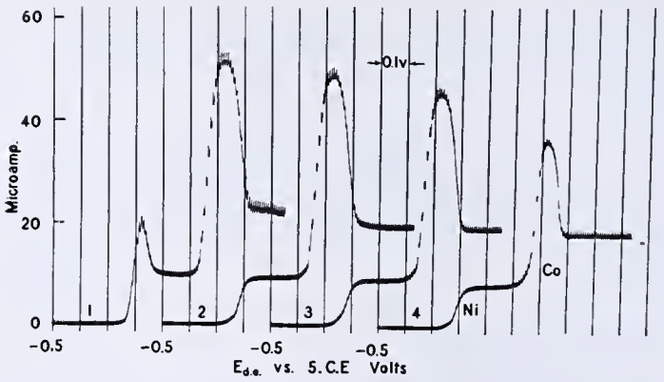

FIGURE 2. INFLUENCE OF GELATIN CONCENTRATION ON NICKEL AND COBALT MAXIMA IN PYRIDINE SOLUTION

0.002 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCl containing 0.5 *M* pyridine. Concentrations of gelatin: (1) none, (2) 0.01, (3) 0.02, (4) 0.05 per cent

3). With concentrations of pyridine less than about 0.2 or 0.3 *M* the double nickel-cobalt wave is very well defined with no maxima, but with increasing concentration of pyridine the cobalt maximum appears and it increases with increasing pyridine concentration.

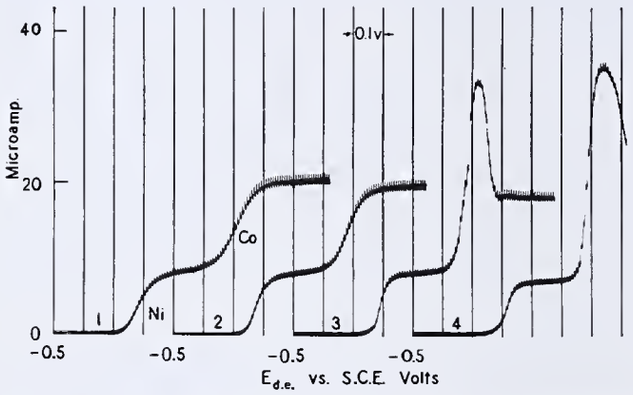

FIGURE 3. INFLUENCE OF PYRIDINE CONCENTRATION ON NICKEL AND COBALT WAVES

0.002 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCl containing 0.05 per cent gelatin. Concentrations of pyridine: (1) 0.05, (2) 0.1, (3) 0.5, (4) 1 *M*

When a pyridinium salt is also present in the pyridine solution, the cobalt maximum is even larger than it was in the foregoing experiments in the absence of the pyridinium ion. This is shown by curve 1 in Figure 4, obtained with a mixture of nickel and cobalt in 0.5 *M* pyridine containing 0.5 *M* pyridinium chloride as supporting electrolyte. However, in the presence of pyridinium ion the cobalt maximum is easily suppressed by 0.05 per cent gelatin or even less, as shown by curve 2.

It will be noted from Figure 4 that the final current rise occurred at a more positive potential in the presence of pyridinium ion. This final current rise at −1.4 volts is due to the reduction of the pyridine, which has been investigated by Shikata and Tachi (*6*). The reduction potential of the pyridine is shifted to a more positive value in the presence of pyridinium salt, owing to the decrease in pH resulting from the buffering effect of the pyridinium ion. However, even in the presence of an equivalent concentration of pyridinium ion, the reduction potential of the pyridine is more negative than that of the cobalt-pyridine complex and does not interfere with the wave of the latter.

The authors have also made a detailed investigation of the nickel and cobalt waves in supporting electrolytes containing thiocyanate, in which medium the waves are slightly more separated than in pyridine solutions (compare Table I). When only nickel is to be determined in neutral solutions, or for the determination of small amounts of nickel in cobalt salts, thiocyanate serves very well as supporting electrolyte. However, a thiocyanate supporting electrolyte is not very suitable for the simultaneous determination of both nickel and cobalt, because in such solutions the diffusion current of the cobalt is not well defined and shows peculiar irregularities, especially in acid solutions or in the presence of ammonium salts. These are the most likely conditions in a practical analysis. For these reasons the use of thiocyanate supporting electrolytes cannot be recommended for the simultaneous determination of nickel and cobalt.

Simultaneous Determination of Nickel and Cobalt in Presence of Iron, Chromium, Manganese, and Copper

Ferric iron is reduced at a potential considerably more positive than the calomel zero, and hence its diffusion current interferes with the waves of practically all other metal ions when it is present in large excess. The authors have found that precipitation as hydrous ferric oxide in pyridine solution affords an excellent separation of relatively large quantities of iron from nickel, cobalt, and copper. Pyridine is a very weak base ($K_b = 1.4 \times 10^{-9}$) and a solution containing equal concentrations of pyridinium ion and pyridine has a pH of about 5.2. At this pH the precipitation of ferric hydroxide and its separation from nickel and cobalt are quantitatively complete.

The outstanding advantage in the use of pyridine is that the precipitation of the iron can be made at a lower pH than when ammonia is used, and hence the possibility of coprecipitation of nickel, cobalt, and copper is greatly lessened.

The separation of small quantities of nickel and cobalt from a relatively large amount of iron by the pyridine method is demonstrated by the polarogram in Figure 5, which was obtained under conditions that simulate the determination of small amounts of nickel and cobalt in steel.

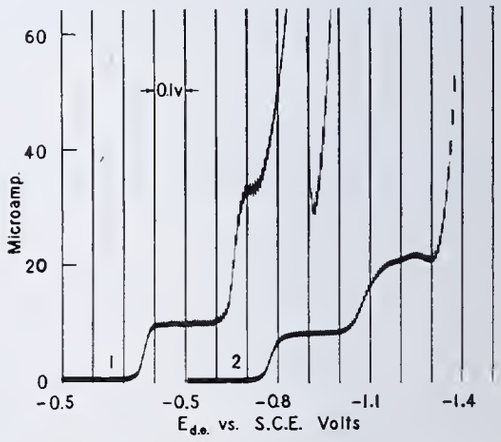

FIGURE 4. INFLUENCE OF GELATIN ON COBALT MAXIMUM IN PYRIDINE SOLUTIONS IN PRESENCE OF A PYRIDINIUM SALT

1. 0.002 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 0.5 *M* pyridinium chloride plus 0.5 *M* pyridine
2. Repeated after adding 0.05 per cent gelatin

Curve 1 was obtained with a solution prepared by diluting 5.00 ml. of 0.01 *M* nickel chloride, 5.00 ml. of 0.01 *M* cobalt chloride, 2.0 ml. of 12 *N* hydrochloric acid, 5.0 ml. of pure pyridine (*ca.* 13 *M*), and 5 ml. of 0.2 per cent gelatin to 100 ml. in a volumetric flask. Hence this solution was 5×10^{-4} *M* in respect to both nickel and cobalt (about 3 mg. of each metal per 100 ml.), 0.24 *N* in pyridinium chloride, 0.4 *M* in pyridine, 0.01 per cent in gelatin, and had a pH of about 5.4. Curve 2 was obtained with a solution prepared in the same way except that 0.3 gram of

iron (as ferric chloride) was added before the addition of the pyridine. The composition of this solution would correspond to about 1 per cent each of nickel and cobalt in a steel. It was not necessary to filter off the precipitated ferric hydroxide; it was simply allowed to settle for a few minutes and a 25-ml. sample of the clear supernatant solution was taken with a pipet and transferred to the polarographic cell for determination of the nickel and cobalt.

The heights of the nickel and cobalt waves were exactly the same after the precipitation of the ferric hydroxide as in the absence of iron, which demonstrates that there was no appreciable coprecipitation of these metals with the iron under these conditions.

For the determination of nickel and/or cobalt in steel a 0.3- to 0.5-gram sample is dissolved in about 5 ml. of concentrated hydrochloric acid in a small beaker, and after addition of a few drops of concentrated nitric acid to oxidize the iron, the solution is evaporated to incipient dryness. The residue is dissolved in 2 ml. of concentrated hydrochloric acid, and transferred to a 100-ml. volumetric flask with 50 to 75 ml. of water. Then 5 ml. of pure pyridine are added, followed by sufficient gelatin solution to give a concentration of 0.01 per cent, and the mixture is made up to the mark and shaken thoroughly. After allowing a few minutes for the ferric hydroxide to settle, a sample of the clear supernatant solution is transferred with a pipet to the polarographic cell and the polarogram is obtained. The dropping electrode is calibrated with known amounts of nickel and cobalt, or with a standard steel sample, under exactly the same conditions.

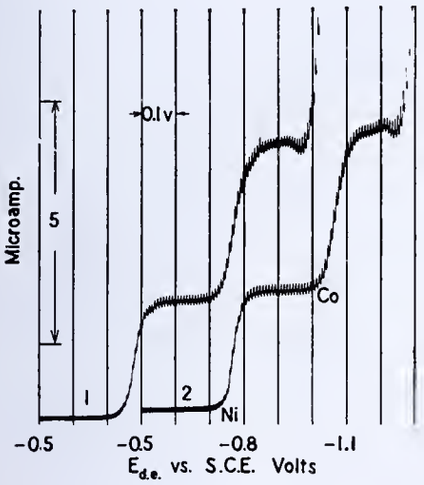

FIGURE 5. SEPARATION OF NICKEL AND COBALT FROM IRON IN PYRIDINE SOLUTION

In the absence of iron, chromic ion is not precipitated in a pyridinium chloride–pyridine solution of pH 5.4, and the chromic-pyridine complex that is formed is reduced to the chromous state with a half-wave potential of −0.95 volt *vs.* the saturated calomel electrode. However, when considerably more ferric iron than chromium was present the chromium was completely coprecipitated with the ferric hydroxide, and the supernatant solution did not show the chromium wave. Hence, moderate amounts of chromium in steel will not interfere with the determination of cobalt and nickel.

Manganous ion in pyridine solution is not reduced below the potential at which pyridine itself is reduced; hence, manganese does not interfere with the nickel-cobalt determination.

Copper in pyridine solution shows two waves, very similar to the double waves obtained in ammoniacal medium (*7*), with half-wave potentials of +0.05 and −0.25 volt *vs.* the saturated calomel electrode (compare Figure 6). The first wave is due to the reduction of the cupric-pyridine complex to the cuprous-pyridine complex, and the second to the reduction of the cuprous complex to the metal (see Figure 6). The copper waves are sufficiently far in advance of the nickel and cobalt waves so that copper does not interfere with the nickel-cobalt determination when its concentration is about the same as (or smaller than) the concentrations of the nickel and cobalt. This is demonstrated by the polarogram in Figure 6 obtained with equal concentrations of copper, nickel, and cobalt in a pyridine solution.

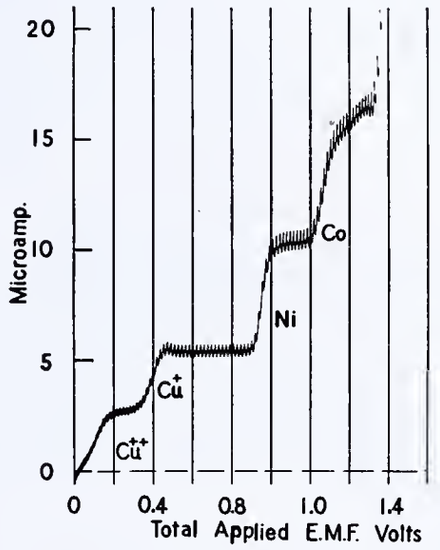

FIGURE 6. SIMULTANEOUS DETERMINATION OF COPPER, NICKEL, AND COBALT IN PYRIDINE SOLUTION

0.001 *M* $CuSO_4$, 0.001 *M* $NiCl_2$, and 0.001 *M* $CoCl_2$ in 0.13 *M* pyridinium perchlorate plus 0.13 *M* pyridine containing 0.02 per cent gelatin

When the concentration of copper is not more than about ten or twenty times that of the nickel and/or cobalt, its diffusion current can be balanced out, and the nickel and cobalt determined, by the "compensation method" (*3*). When a larger excess of copper is present, the bulk of it must be removed before nickel and cobalt can be determined. In such a case it is not necessary to remove the copper completely, but only to reduce its concentration to the same order of magnitude as the concentration of the nickel and cobalt.

Determination of Small Amounts of Nickel in Cobalt Compounds

The use of pyridine or thiocyanate supporting electrolytes makes possible the rapid and accurate determination of small amounts of nickel present as an impurity in cobalt and cobalt compounds. This is illustrated in Figure 7.

Curve 1 in this polarogram was obtained from a solution prepared by dissolving a 3.00-gram sample of reagent quality cobalt sulfate heptahydrate in about 50 ml. of water in a 100-ml. volumetric flask, adding 2 ml. of 12 *N* hydrochloric acid, 5 ml. of pure pyridine, and 5 ml. of 0.2 per cent gelatin, and diluting to 100 ml. A 75.0-ml. portion of this solution was used for obtaining curve 1, which shows a well-defined wave of the nickel impurity. After the first curve was obtained, 4.00 ml. of a 9.24×10^{-3} *M* nickel chloride solution were added to the solution in the cell and curve 2 was recorded. The diffusion current of the nickel is directly proportional to its concentration, and hence from the increase in the height of the nickel wave resulting from the addition of the standard nickel solution the original concentration of nickel can be easily computed.

Let V = original volume of solution in cell
v = volume of standard nickel solution added
C_1 = original molar concentration of nickel
$C_{Std.}$ = molar concentration of standard nickel solution
i_1 = original diffusion current of nickel
Δi = increase in diffusion current resulting from standard addition
ΔC = increase in concentration due to standard addition

We then have the following relations

$$C_1 = \frac{i_1}{k} \quad (1)$$

and

$$\Delta C = \frac{\Delta i}{k} = \frac{v}{V + v} C_{\text{Std.}} \quad (2)$$

or

$$k = \frac{\Delta i(V + v)}{vC_{\text{Std.}}} \quad (3)$$

Therefore,

$$C_1 = \frac{i_1 v C_{\text{Std.}}}{\Delta i(V + v)} \quad (4)$$

In the present experiment, i_1 = 1.97 microamperes, Δi = 1.98 microamperes, V = 75.0 ml., v = 4.00 ml., and $C_{\text{Std.}}$ = 9.24×10^{-3} M. Therefore the original concentration of nickel was

$$C_1 = \frac{1.97 \times 4 \times 9.24 \times 10^{-3}}{1.98 \times 79} = 4.65 \times 10^{-4} M$$

This corresponds to 0.091 per cent of nickel, or 0.44 per cent of nickel sulfate heptahydrate, in the original sample of cobaltous sulfate heptahydrate.

This result is believed to be accurate to about ±3 per cent, and is at least as accurate as the determination of this small amount of nickel would be by the classical dimethylglyoxime procedure. Furthermore, the polarographic method is more rapid and less troublesome than the dimethylglyoxime method.

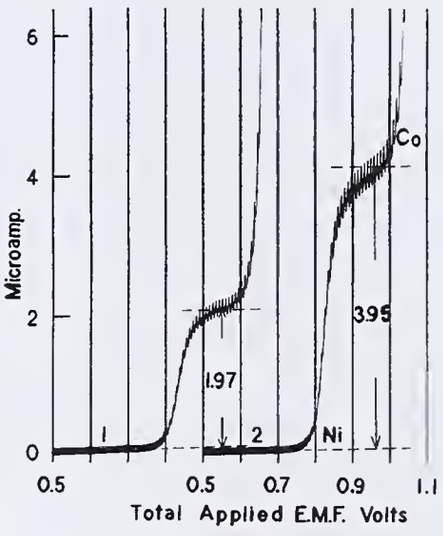

FIGURE 7. DETERMINATION OF NICKEL IMPURITY IN COBALTOUS SULFATE

It will be noted from Figure 7 that in the presence of a large excess of cobalt the reduction of cobalt begins before the diffusion current of the nickel has become entirely constant. For this reason it is necessary to use a standardized technique for measuring the nickel diffusion current. The authors have found that reliable and consistent results are obtained when the wave height of the original solution and the wave height after addition of the standard nickel solution are both measured at the same potential as shown in Figure 7. In the present case the optimum potential for this measurement is 0.10 to 0.12 volt beyond the half-wave potential. Correction for the "residual current" is made by extrapolation as indicated by the dotted lines.

For maximum precision the amount of standard nickel solution added should be sufficient just about to double the height of the original nickel wave. After a little experience with a given capillary, the amount to add can be readily estimated from the height of the original wave. Furthermore, in routine applications of this method, with the same capillary and with all other conditions constant, the calibration by addition of standard nickel solution would have to be made only once, and in subsequent analyses the concentration of nickel could be read directly from the height of the original nickel wave.

The application of this method to other cobalt compounds will be obvious. For determining nickel in metallic cobalt, cobalt oxide, etc., a sample of the material would be dissolved in hydrochloric acid, excess pyridine added, and then the foregoing procedure followed. Incidentally, the removal of nickel from cobaltous chloride by recrystallization from water is very ineffective. Only 35 per cent of the original amount of nickel (0.15 per cent) present in a sample of cobaltous chloride was removed by two recrystallizations.

The foregoing procedure can also be used for the rapid determination of small amounts of copper, either alone or simultaneously with nickel, in cobalt compounds.

Summary

In supporting electrolytes containing pyridine or thiocyanate the half-wave potential of nickel is 0.3 volt more positive than that of cobalt, and the excellent separation of the two waves permits the simultaneous determination of both metals. The use of a supporting electrolyte containing pyridine is preferable to one containing thiocyanate, because with the latter the diffusion current of cobalt shows peculiar irregularities, especially in acid solutions or in the presence of ammonium salts.

A sharp separation of ferric iron from small amounts of nickel, cobalt, and copper by precipitation as hydrous ferric oxide is obtained with a supporting electrolyte of pH equal to about 5.4 containing equal concentrations of pyridine and a pyridinium salt. Nickel and cobalt are not coprecipitated with the hydrous ferric oxide under these conditions, and the method is well suited to the simultaneous determination of nickel and cobalt in steel. Moderate amounts of chromium in steel are completely coprecipitated with the hydrous ferric oxide, and hence do not interfere with the determination of nickel and cobalt. Manganese and small amounts of copper do not interfere with the nickel-cobalt determination. Copper gives a double wave at +0.05 and −0.25 volt *vs.* the saturated calomel electrode in pyridine solutions, and when present in large excess the bulk of it must be removed prior to the nickel-cobalt determination.

Small amounts of nickel impurity in cobalt compounds can be rapidly and accurately determined in a supporting electrolyte containing pyridine or thiocyanate.

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Colorimetric Determination of Phosphorus in Iron Ore

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COLORIMETRIC methods have been described for the determination of phosphorus in iron and steel.

Zinzadze (*5*) described a method for determining phosphorus in the presence of silica, iron, and nitrates, but because of the sensitive nature of the molybdenum blue and the time necessary to develop it, it does not seem applicable to iron ore. Murray and Ashley (*3*) and Bogatzki (*1*) described methods for the determination of phosphorus in iron and steel by converting it into a yellow complex phosphovanadomolybdate, which according to Misson (*2*), who first suggested this method, has the formula $(NH_4)_3PO_4.NH_4VO_3.16MoO_3$. The color is very stable and undergoes no appreciable color change after 14 days. Murray and Ashley (*3*) used the Pulfrich photometer and worked at 430 mμ. They found, however, that high silica interfered with the determination, because of the formation of yellow silicomolybdic acid.

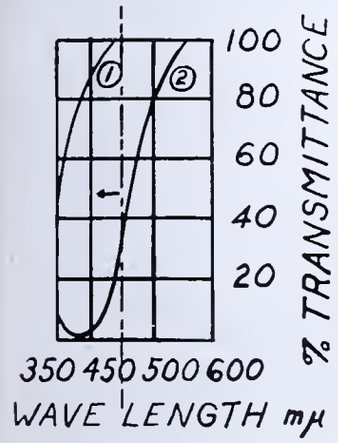

FIGURE 1. TRANSMITTANCE CURVES
1. Ferric perchlorate
2. Ferric chloride

Of all the methods studied this modification of Misson's method seemed the most promising, but it has two serious objections: (1) Iron ores are usually high in silica as compared to irons and steels, and therefore the formation of silicomolybdic acid would interfere seriously; (2) the iron content of ores varies considerably more than that of irons and steels, and since ferric chloride shows a large absorption of light at 430 mμ, the interference of iron would be serious. The method which is described here eliminates both of these difficulties.

Experimental

In attempting to determine phosphorus by measuring the intensity of a reduced solution of molybdenum blue, it was found that the color which had once been developed could not be diluted, since its intensity depended on the pH, and that a variation in the concentration of stannous chloride added as a reducing agent caused a change in the color. Moreover, considerable time was required for the maximum color to develop. For these reasons this method was abandoned.

It was necessary to find a reagent which would completely remove the silica and would also remove the yellow color of ferric iron. The hydrochloric acid solution of the ore was therefore evaporated with perchloric acid, which dehydrated the silica and rendered it easily filtered (*4*). The resulting ferric perchloric solution is practically colorless and at the same time the acidity of the solution is correct for the formation of the yellow complex.

Apparatus

A Coleman Model 10 regional spectrophotometer was used to measure the amount of monochromatic light transmitted through the yellow solution. The width of the spectral band used was 30 mμ. A blank containing iron but no phosphorus was first placed in the path of the light and the instrument was balanced. This was then replaced by the sample and the percentage of light transmitted as compared to the blank was measured.

It was necessary to allow the lamp to warm up for 10 minutes before making any measurements.

The effect of perchloric acid in removing the yellow color characteristic of ferric chloride is shown in Figure 1, in which curve 2 shows the transmittance for ferric chloride solutions and curve 1 for ferric perchlorate. It will be noted that conversion of iron into perchlorate has moved the region of maximum transmittance into the ultraviolet and this colorless solution now offers no interference in measuring the transmittance of the yellow phosphovanadomolybdate complex.

The nearer the ultraviolet region is approached the more interference by iron can be expected. On the other hand, if 550 mμ is approached a difference in the concentration of phosphorus makes very little difference in the percentage of light transmitted, as shown by Figure 2. Therefore, 450 mμ was chosen as the correct wave length because it is as near to the ultraviolet as possible without interference from the iron content.

Effect of Varying the Concentration of Perchloric Acid

Past a certain minimum the maximum color was developed at a low acid concentration. If more than 13 ml. of perchloric acid was used the color did not develop a full maximum. Less than that amount allowed the formation of a precipitate on the addition of ammonium molybdate.

The amounts of ammonium vanadate and ammonium

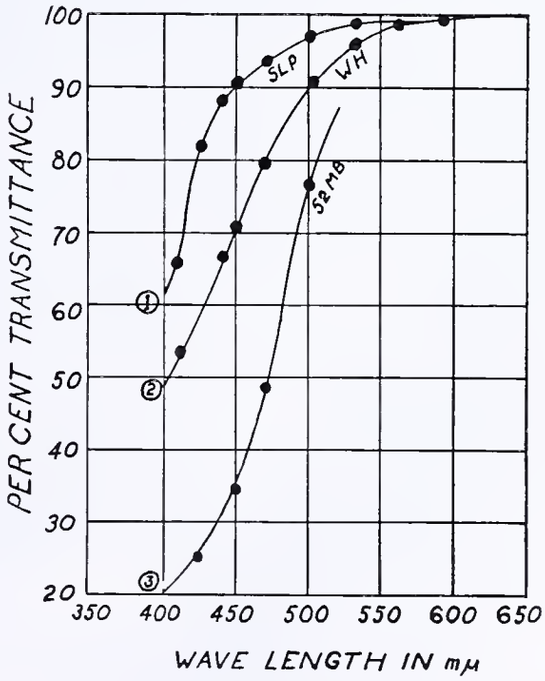

FIGURE 2. TRANSMITTANCE–WAVE LENGTH CURVES FOR THREE IRON ORES
Phosphorus (1) 0.019 per cent, (2) 0.0705 per cent, (3) 0.243 per cent

[1] Present address, Oliver Iron Mining Company, Hibbing, Minn.

molybdate were always in excess of the calculated values for a 0.500-gram sample high in phosphorus.

Reagents

AMMONIUM VANADATE SOLUTION. To 2.350 grams of ammonium metavanadate dissolved in 500 ml. of hot distilled water add 20 ml. of 1 to 1 nitric acid and dilute to 1 liter.

AMMONIUM MOLYBDATE SOLUTION. Dissolve 50 grams of molybdic acid in a mixture of 200 ml. of distilled water and 40 ml. of concentrated ammonium hydroxide. Filter the solution, boil the filtrate 20 minutes, and dilute to 500 ml.

TABLE I. DATA USED IN PLOTTING TRANSMITTANCE-CONCENTRATION CURVE

Sample	Phosphorus, Per Cent	Transmittance at 450 mμ
SLP standard, OIMC[a], National Bureau of Standards	0.019	90.6
11 R, OIMC[b]	0.030	87.0
28 Cargo, OIMC[b]	0.035	84.4
114 S, OIMC[b]	0.049	78.3
WH standard, OIMC, and other laboratories	0.0705	70.5
Soudan standard, OIMC, and other laboratories	0.118	57.1
39 MB, OIMC	0.149	50.8
03 MB, OIMC	0.196	40.2
29 MB, OIMC	0.224	35.9
52 MB, OIMC	0.243	34.4

[a] Oliver Iron Mining Co., Hibbing, Minn.
[b] Samples checked at OIMC for soluble and insoluble phosphorus.

Procedure

Weigh out a 0.5-gram sample of iron ore, place it in a 150-ml. beaker, add 10 ml. of concentrated hydrochloric acid, and heat the covered solution on a hot plate until the ore is dissolved. It may be necessary to add more hydrochloric acid. When the ore is in solution, evaporate the solution almost to dryness (do not bake), and add 13 ml. of 70 to 72 per cent C. P. perchloric acid. Boil the mixture on the hot plate until the dark ferric solution has changed to a straw yellow color. This usually takes 4 or 5 minutes, and at this point the sample should be fuming strongly. (If a Méker burner is used instead of a hot plate, the time required to reach fumes of perchloric acid can be reduced to 2 or 3 minutes.) Cool the solution slightly and add from a pipet 10 ml. of ammonium vanadate solution. Boil for about 30 seconds to remove any chlorine present, remove the beaker from the hot plate, and place it in a pan of cold water until it can be held in the hand. Wash off the cover glass and sides of the beaker with a little distilled water and filter the solution into a 100-ml. volumetric flask. Wash the beaker and paper with 15 ml. of ammonium molybdate solution, added from a small graduate, shake thoroughly until the precipitate that first forms is dissolved, and dilute to the mark. Shake the flask thoroughly and allow it to cool either by standing or by immersion in water until it is at approximately room temperature.

Place the sample in a Coleman spectrophotometer, measure the transmittance at 450 mμ compared to the blank, and from the curve read off the percentage of phosphorus. If the ore does not dissolve too slowly, the entire procedure may be completed in half an hour.

The blank is made by weighing out 0.255 gram of pure iron wire and running it exactly as the regular sample was run.

Figure 3 is the curve obtained by applying this method to ten ores in which the phosphorus had been carefully determined by a standard method, and Table I shows the data from which it was constructed.

Since the concentration of a solution is proportional to the logarithm of the intensity of the transmitted light, the logarithm of the transmittance at 450 mμ is plotted against the concentration of phosphorus. The curve passes almost through the 100 per cent transmittance point, which indicates that the blank is theoretically correct.

Precautions

The evaporation with perchloric acid should not be carried beyond the color change because acid will be lost by evaporation and if the acidity of the solution is too low the precipitate first formed on the addition of ammonium molybdate will not dissolve on shaking.

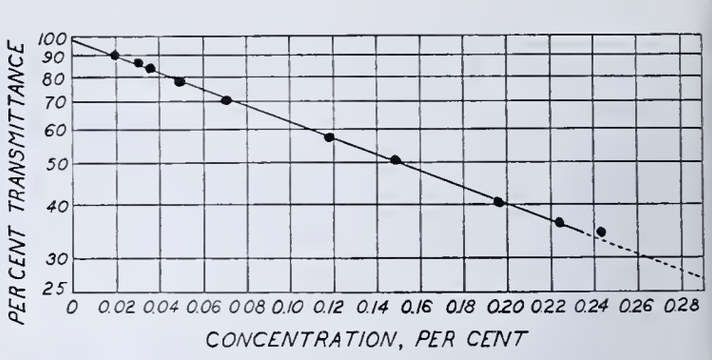

FIGURE 3. CONCENTRATION OF PHOSPHORUS *vs.* TRANSMITTANCE

After the development of the color the solution should be allowed to stand at least 4 minutes before measuring the per cent of transmittance.

If chlorine is not boiled off, it has a tendency to dissolve the filter paper and also causes variations in the intensity of the developed color.

Stannous chloride cannot be used to hasten the solution of the ore in hydrochloric acid because it causes a turbidity.

Reasonable care should be taken that no organic matter comes in contact with the boiling concentrated perchloric acid during the evaporation.

TABLE II. PRECISION OF METHOD

	Transmittance at 450 mμ %	Deviation %
WH standard	70.2	−0.3
	69.1	−1.5
	70.4	−0.1
	71.4	1.1
	70.6	0.1
	70.2	−0.3
	70.7	0.2
	71.3	0.8
	Av. 70.5	
SLP standard	90.6	0
	90.8	0.2
	91.0	0.4
	91.0	0.4
	89.8	−0.8
	90.5	−0.1
	Av. 90.6	

Precision of the Method

Of the ten samples used in constructing the curve, two are selected to show the precision of the method (Table II). By the method of least squares the average deviation for the WH samples is calculated to be approximately 0.6 and that of the SLP samples 0.35. The two deviations are typical of all samples run.

On examining Figure 3 it is seen that for a sample low in phosphorus 1 per cent in transmittance corresponds to 0.002 per cent of phosphorus, and for a sample high in phosphorus 1 per cent transmittance corresponds to approximately 0.006 per cent of phosphorus. Thus for the WH standard the deviation would be, for high phosphorus, $0.006 \times 0.6 = 0.0036$ per cent phosphorus, and for low phosphorus, $0.002 \times 0.6 = 0.0012$ per cent phosphorus. The average is 0.0024. For the SLP standard the corresponding values are 0.0021 and 0.0007 per cent, the average being 0.0014 per cent. The average for both samples is, in round numbers, 0.002 per cent. This precision is about the same as that obtained by usual methods.

Summary

The colorimetric determination of phosphorus in iron ore by the phosphovanadomolybdate method has been greatly improved by converting the iron to colorless ferric perchlorate. This not only removes interfering silica but shortens the time required, and makes possible the use of the more favorable

wave length of 450 mμ. The transmittance at this wave length is determined and from a standard curve the percentage of phosphorus is calculated.

Acknowledgment

The authors are indebted to Eberbach & Son Co., Ann Arbor, Mich., for the use of the spectrophotometer.

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Basic Sulfates of Iron and Aluminum in Analytical Separations

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AS DESCRIBED in a previous paper (*3*), a solution of ferric sulfate was used to form a basic ferric alum with potassium which was thereby separated from cesium. In a similar manner, after the addition of potassium sulfate an excess of iron can be separated from divalent metals, which are then determined by the usual methods. In this procedure a basic sulfate of iron and potassium is formed at a definite pH and at steam bath temperature, 90° C. The precipitation of iron is nearly complete, while divalent metals remain in solution. A small part of any aluminum present is also precipitated.

Mellor (*7*) mentions as products of the hydrolysis of ferric alum a few basic sulfates of iron and potassium which vary in composition and are microcrystalline. Krueger (*5*) obtains a basic sulfate of iron and ammonium in separating iron from cobalt, nickel, zinc, and manganese. He fails to consider the presence of aluminum and completes the separation of iron as a basic acetate. Ardagh and Bongard (*1*) obtain good separations of nickel and zinc from iron and aluminum in a small volume of solution containing a little hydrochloric acid and 5 grams of ammonium chloride, to which strong ammonia is added in excess. Noyes and Bray (*9*) separate 2 to 50 mg. of nickel or zinc from 100 mg. of iron very satisfactorily by the above procedure, but with cobalt 1 mg. in 50 is caught in the precipitate of iron. This separation is troublesome and requires repetition, especially when excessive quantities of iron and aluminum are present. Lundell and Knowles (*6*) find that nickel only may be satisfactorily separated by the Blum method in a single precipitation. The writer has observed that the separation from aluminum is by far the more uncertain. Nickel only can be separated in a faintly acid solution containing ammonium sulfate.

Aluminum, like iron, forms a basic sulfate with potassium. A patent on basic alum has been issued to Fleischer (*4*), who heats a solution of alum above 60° C. in a continuous system. Titanium forms no double sulfate, as tested by experiment. According to Mellor (*8*) magnesium, zinc, or manganese may enter the basic sulfate molecule, which resembles alunite in composition but contains the same proportion of potassium sulfate as ordinary alum. Cobalt also forms a double sulfate with aluminum. Aluminum may be separated from beryllium by this method satisfactorily without the use of ammonium sulfate. Britton (*2*) obtains a 90 per cent separation of aluminum from beryllium as potassium alum.

Procedure

Treat a slightly acid solution of ferric sulfate, free from fluoride, chloride, and nitrate, with potassium acid sulfate and partially neutralize with dilute ammonia until a precipitate begins to persist. Heat this solution for several hours in a flask lowered directly into the steam bath; a dense, microcrystalline, orange-red precipitate is produced. Potassium sulfate yields a less soluble precipitate than the ammonium salt. The presence of titanium or phosphate renders subsequent filtration slow and the filtrate yellow with too much iron. In such a case, further neutralize the filtrate and repeat the hydrolysis. About 5 mg. of iron remain in solution. If appreciable quantities of titanium are present, first hydrolyze at 0.1 *N* acidity. Proceed in like manner for phosphate after adding titanium sulfate.

Determination of Zinc and Nickel. Place the solution of iron and zinc sulfate in a 500-ml. Kjeldahl flask, add potassium acid sulfate equal in weight to the ferric oxide probably present, dilute to 300 ml., and slowly add dilute ammonia until a slight permanent precipitate remains. Place a small funnel in the neck of the flask and heat to 90° C. in the steam bath for at least 4 hours, or overnight. Hydrolysis with a wired-in stopper yields a more granular basic sulfate. Filter while hot. The filtrate should be colorless and have a pH close to 2.8, just yellow to thymol blue. Evaporate the filtrate and wash water to 150 ml. Filter off any precipitate of iron, add methyl orange, and neutralize until the color just remains red, at a pH of 3.1, which is about right for the precipitation of zinc sulfide. Transfer the solution to an Erlenmeyer flask, add 2 ml. of 5 per cent mercuric chloride solution, and precipitate zinc and mercury with hydrogen sulfide. Ignite the filtered and washed sulfides and weigh as zinc oxide in the usual way. If cobalt or nickel was originally present the precipitation of zinc may require repetition.

After removing the greater part of the iron as above, concentrate the filtrate and wash water to 300 ml., add methyl orange, and neutralize the filtrate until faintly red. Add 5 grams of ammonium sulfate and hydrolyze in steam for at least 4 hours. This second hydrolysis removes more iron, together with half of any aluminum present. Ammonium sulfate keeps nickel in solution. Concentrate the filtrate from aluminum to 150 ml., add 2 grams of tartaric acid, and precipitate nickel with dimethylglyoxime in the usual way.

The hydrolysis of ferric sulfate begins at about pH 1.2 and some iron remains in solution at pH 3.4. This figure was first calculated, then verified after the hydrolysis by direct determination in a pH meter. As the pH is increased to 3.8 the basic sulfates become slimy and filter slowly. At pH 3.8 the solubility for either iron or aluminum is still about 5 mg.

The hydrolysis of aluminum sulfate begins at about pH 2.8 and is one-half complete at pH 3.1. The solubility at 3.4 in terms of aluminum oxide is increased to 35 mg. in the presence of 10 grams of ammonium sulfate.

In the analysis of meteorites, stainless steel, and some minerals, a few per cent of the divalent metals are present with large percentages of iron or both iron and aluminum. Numerous quantitative tests were made of the behavior of such combinations involving cobalt, nickel, zinc, or beryllium.

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Biometry in the Service of Biological Assay

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SOME chemists may wonder how biometry, or statistics applied to biology, is involved in the biological assay of drug potency. The biometrician or statistician on the other hand may be equally puzzled that the pharmacologist and chemist have been willing to work for so long without his help. Sometimes he is asked to evaluate experimental data after the laboratory work has been completed and the possibility of modifying the original technique has passed. This is no more logical than to hold an analytical chemist responsible for determining the original sugar content of a sirup which may have partly fermented before it reaches him, owing to improper preservation. To realize its full possibilities, statistical control must also start from the beginning, with the original experimental design. Then modern statistical methods may be expected to give more precise results with fewer animals and a measure of just how far a given determination should be trusted, whether it occurs in a routine assay or in developing a new medicinal product.

For a quantitative approach to biological assay, one must first adjust himself to an order of variation in the original measurements far greater than that customary in analytical chemistry. Because in most cases this variation agrees closely with the normal curve of error, many advantages are at once available to the biologist. One distinction must be emphasized. Consistency with the normal curve of error does not imply a faulty technique on the part of the experimenter or a mistake in his measurements. This is an inherent characteristic of many animal populations and the more carefully the animals are selected and handled and the more accurately each response is measured, the more nearly they will adhere to the normal curve of error.

All-or-None Effects of Drugs

The normal curve can be demonstrated most readily from measurements of the toxicity of the digitalis glucosides to individual cats by slow intravenous infusion.

In a long series of tests at the Lilly Research Laboratories, a single worker infused the laboratory standard of digitalis or Digiglusin simultaneously into each of 4 cats until its heart stopped. The results on 52 of these groups of 4 have been reported recently by Bliss and Hanson (*3*). In their group No. 2, for example, the 4 replicated analyses gave the following lethal doses: 29.6, 25.8, 29.2, and 19.9 cc. per kg. of cat. An analytical chemist might consider the first and third replicates in satisfactory agreement, the second questionable, and the fourth probably an error in analysis. Let us take another set of 4 from the same series, No. 52, for example, where the 4 replicates gave 28.1, 26.2, 22.5, and 34.4 cc. per kg., respectively. Here it would be more difficult to separate the "good" from the "bad" determinations and it is evident that a rule-of-thumb approach is not of much use. Instead we will assume that mistakes in titrating the individual cats were negligible when compared with the variations in their susceptibility to the drug and center our attention upon the nature of this variation. For such a study selected groups of 4 are clearly less satisfactory than an analysis based upon the entire series without selection.

The first question concerns the unit of computation, for any unit which may be adopted commits us at once to specific biological assumptions as to the nature of digitalis action. The better the basis for these assumptions, the greater is the chance that the computation will lead to a satisfactory conclusion. Digitalis acts directly upon heart tissue and in relating the quantity of drug which poisons the heart of each individual to its gross body weight, it is assumed that the mass of specific heart tissue is directly proportional to that of the rest of the animal. For the present data this has proved to be a valid assumption. However, if the dose in cubic centimeters of extract per kilogram is used directly in arithmetic units, we assume further that the effect of a given increase in dosage is constant, regardless of an individual's inherent susceptibility or of previous dosage. The weight of pharmacological evidence shows that to obtain a uniform increment in effect, the dosage of most drugs must be increased by a constant proportion or percentage. It is better, therefore, to base our analysis upon this more likely assumption by transforming the just-lethal dose for each cat to logarithmic units. If the assumption is ill-founded, statistical analysis will disprove it.

Only 4 cats were tested in parallel, although the 52 assays covered a period of 18 months. More often than not, the susceptibility of animals to the same sample of drug varies from one test day to the next. To control these unpredictable variations pharmacologists have adopted standard preparations for many drugs with which new samples can be compared by running both at the same time. If the reaction of cats to digitalis were to vary similarly, the means of the groups of 4 animals should vary more than would be expected from the variation within groups of 4. This possibility has been tested by one of the most flexible and valuable of all statistical tools, the analysis of variance. The differences between the log-dose per kilogram for each individual cat and the general mean for all 52×4 or 208 cats are squared and summed to obtain a total sum of squares which is then separated into two portions, that due to differences between the groups of 4 and that arising from variations in susceptibility within these groups. In terms of squared deviations these sources of variation are additive and we have the separation shown in Table I, where the mean square or variance

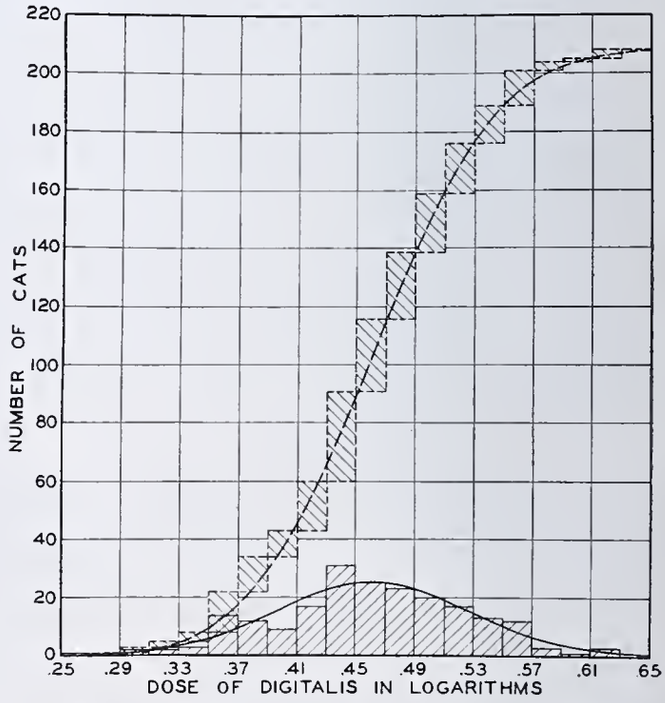

FIGURE 1. VARIATION IN SUSCEPTIBILITY OF INDIVIDUAL CATS TO DIGITALIS AFTER CORRECTION FOR DIFFERENCES BETWEEN DAYS

From Bliss and Hanson (*3*)

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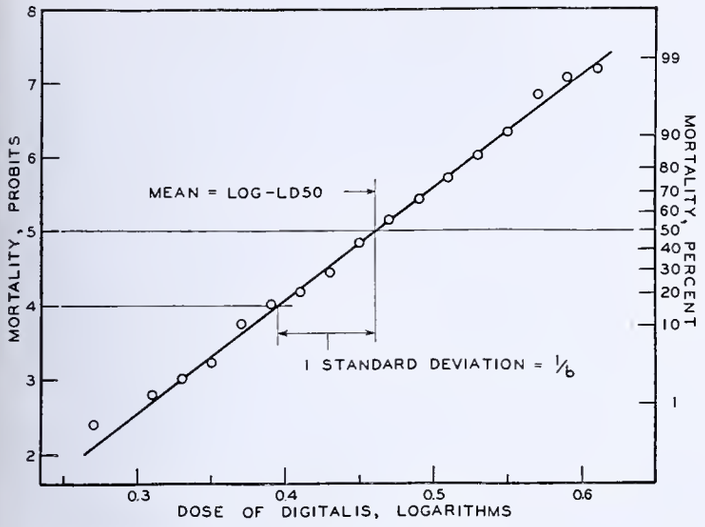

FIGURE 2. CUMULATIVE CURVE OF FIGURE 1 PLOTTED IN LINEAR FORM

between assays is 2.44 times as great as that within assays or groups of 4, a difference which would not be expected by chance alone even once in 1000 similar experiments. By avoiding the larger variation between groups and always comparing an unknown with a standard upon cats tested in parallel, it is apparent that fewer animals will suffice in assaying the potency of an unknown in terms of the laboratory standard of digitalis for any required degree of precision. The same principle holds throughout all biological assay.

TABLE I. PARTITION BY ANALYSIS OF VARIANCE OF VARIATIONS IN TOXICITY OF DIGITALIS TO CATS

[In terms of deviations from the mean log-dose in cc. per kg. of cat; data from Bliss and Hanson (*3*)]

Variation	Degrees of Freedom	Sum of Squares	Mean Square or Variance	Variance Ratio	Standard Deviation
Between assays	51	0.52992	0.010391	2.44	0.10193
Within assays	156	0.66478	0.004261	1	0.06528
Total	207	1.19470			

We are now ready to examine the nature of the variation in susceptibility between cats. The differences between the group means and the general mean have been multiplied by the ratio of the standard deviation ($= \sqrt{\text{mean square}}$) within assays to that between assays and these corrections added to or subtracted from each individual measurement to bring all of the determinations to a common basis. Then they could be studied just as if all 208 cats had been run simultaneously with the same precision as was obtainable from groups of 4. The number of cats dying at adjusted doses within each of 20 equal log-dose intervals has been plotted along the base of Figure 1 in a block diagram. The smooth curve drawn through the tops of these blocks is the computed normal curve of error. It evidently fits well, as can be shown also by statistical test. The fact that the variation in susceptibility is symmetrical and normal when measured in terms of the logarithm of the just-lethal dose justifies the original assumption underlying the transformation to logarithms.

By a simple transformation the "frequency distribution" along the base of Figure 1 can also be plotted as a straight line and in this form it is comparable to the much larger group of bioassays for which the just-lethal dose cannot be measured individually. The data are converted first to a cumulative form by moving the block for each dosage interval along the base of Figure 1 vertically upward until its lower edge is contiguous with the upper edge of the next preceding block. They then describe a symmetrical sigmoid curve conforming to the cumulative normal curve of error which intersects them. From the intersection of each observed cumulative number of cats with the sigmoid normal curve in Figure 1, one could interpolate in abscissal units a log-dose predicted from the response. Plotting these predicted log-doses against those observed experimentally should define a straight line if the data conform to the normal curve.

The same result can be accomplished more easily. Frequencies are changed to the percentage of individuals reacting at all dosages up to and including each successive log-dose. Then these percentages of effect can be transformed by tables based upon the normal curve to hypothetical "dosages" in units of the standard deviation, such as the "normal equivalent deviations" of Gaddum (*7*) or the "probits" of Bliss (*1*). When these are plotted against the corresponding observed log-doses, a straight line should result as in the data replotted in Figure 2 from Figure 1. When the points fall along a straight line, the mean of the observed log-doses is the log-LD50 or the log-dose for 5 probits or 50 per cent kill and the reciprocal of their standard deviation fixes the slope of the line from which the log-dose can be interpolated for any required percentage effect or vice versa.

In most assays depending upon an all-or-none reaction, the just-effective dose cannot be determined for each individual and separate lots of animals are treated uniformly with predetermined dosages of drug. The positive reactors in each lot, expressed as a percentage, include of course those that would have reacted at all smaller doses. Hence the initial data are recorded in a cumulative form and the relation between percentage effect and log-dose of drug is typically a symmetrical sigmoid curve. A good example has been reported by Morrell, Chapman, and Allmark (*10*) on the therapeutic assay of neoarsphenamine from the incidence of negative blood smears in the male rat. Six series have been adjusted for differences in over-all susceptibility, plotted in Figure 3, and fitted by the cumulative normal curve. Statistical test shows that these observations agree with the curve rather better than would be expected by chance. Yet even the present amount of scatter would inject an appreciable subjective error in a sigmoid curve drawn through these points merely by inspection. By transforming percentages to probits the same data can be plotted as shown in Figure 4 and fitted by the simplest curve of all—a straight line. Moreover, the reliability of the curve or of the log-LD50 or

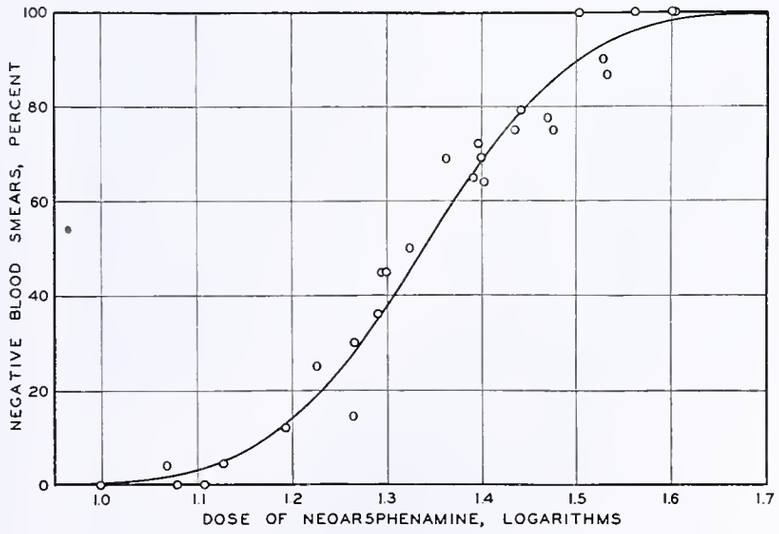

FIGURE 3. SIGMOID DOSAGE-EFFECT CURVE FOR THERAPEUTIC ACTION OF NEOARSPHENAMINE IN MALE RATS

From data of Morrell, Chapman, and Allmark (*10*). Six independent curves superimposed by adjustment of dose.

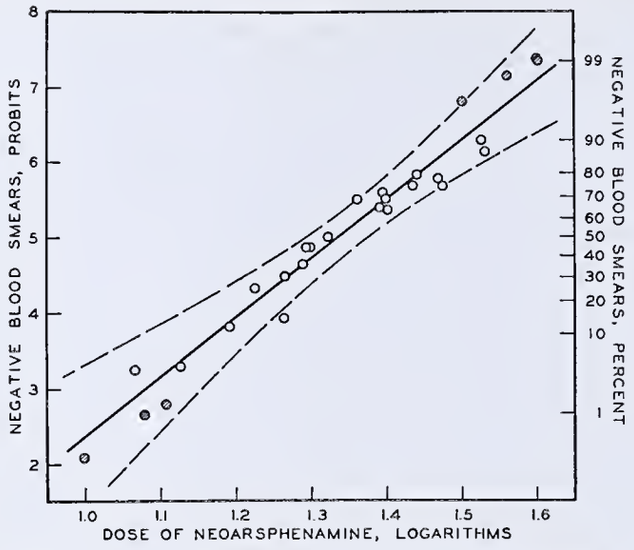

FIGURE 4. DOSAGE-EFFECT CURVE OF FIGURE 3 PLOTTED IN LINEAR FORM

Zone of error, designated by broken lines, shows expected precision for one of six component series.

of any other point upon it can be estimated for any given odds, as shown in the diagram by the broken parabolas. Here they indicate the average limits which would apply to one of the 6 series, involving 5 lots each of 20 to 25 rats, and would enclose the true curve in 19 out of 20 determinations ($P = 0.05$).

Since the biological indicators of drug action vary in an orderly manner, we can use these curves and the techniques for their computation to measure relative potency. Two or more dosages of both standard and unknown are administered and the data plotted separately on linear coordinates. If the unknown produces the same biological effect as the standard, their respective curves should be parallel within the limits of the experimental error; if the unknown has the same potency as the standard, the two parallel lines should coincide, again within the limits of the experimental error. When the curves do not coincide but are parallel, the horizontal distance between them, of course, is the same at all levels of effect and, being a difference on a logarithmic scale of dosage, measures in logarithms the ratio of their potencies. By adjusting the concentration of the unknown, its potency can be made equal to that of the reference standard. Since the error of the ratio is computed as an integral part of the assay, the expected variation in this potency will be known.

A convenient example has been given by Miller, Bliss, and Braun (*9*) in an assay comparing the relative potency of digitalis in the frog when injected intramuscularly as compared with standard injection into the lymph sac. Three doses were administered by each route with the results shown in Figure 5. The log-ratio of potencies and its standard error, designated by the symbols $M \pm s_M$, were 0.319 ± 0.043. Changing the route of injection had the same effect, therefore, as increasing the potency of the solution of digitalis by 209 ± 20 per cent when assayed by the 1-hour method. The odds are 2 out of 3 that the true potency did not differ more above or below the estimated value than the standard error. By successive repetitions of the assay, this precision could be increased to any point which may be required.

Graded Response

So far, we have been considering only all-or-none effects of drugs, where a given reaction is either present or absent. Although perhaps death occurs more frequently than other end points, the method has been applied to many criteria, such as convulsions, systolic standstill of the heart, the presence of cornified cells in the vaginal epithelium, negative blood smears, and survival in a therapeutic test. Another large group of assays depends upon a graded response, where the extent of the reaction varies with the dose. Examples are the hypoglycemic response of the rabbit to insulin, the height of contraction of guinea pig uterine muscle to posterior pituitary extract, the level of serum calcium in the dog following treatment with parathyroid extract, and the growth of depleted mice under different dosages of vitamin A. Since each individual reaction is quantitative rather than qualitative, it contributes more information per animal than in the preceding type. The statistical methods suitable for an estimate of $M \pm s_M$ differ in many respects.

The first problem is to obtain a criterion of graded response which will plot as a straight line against the logarithm of the dose. Unlike the all-or-none reaction, no comprehensive transformation has been discovered as yet that is applicable to a wide variety of responses. Since the effect that it is practicable to measure is usually an empirical composite of several factors, which differ widely from one type of drug to another, a transformation as general as Gaddum's N. E. D. or the probit is relatively improbable. When graded reactions are studied over a wide enough range of dosages, they tend to flatten out toward a lower limit or floor and toward an upper limit or ceiling, leading to a sigmoid form. Frequently, however, an extended central section does not differ appreciably from a straight line or other factors intervene, so that the curved portion approaching a limit is never reached.

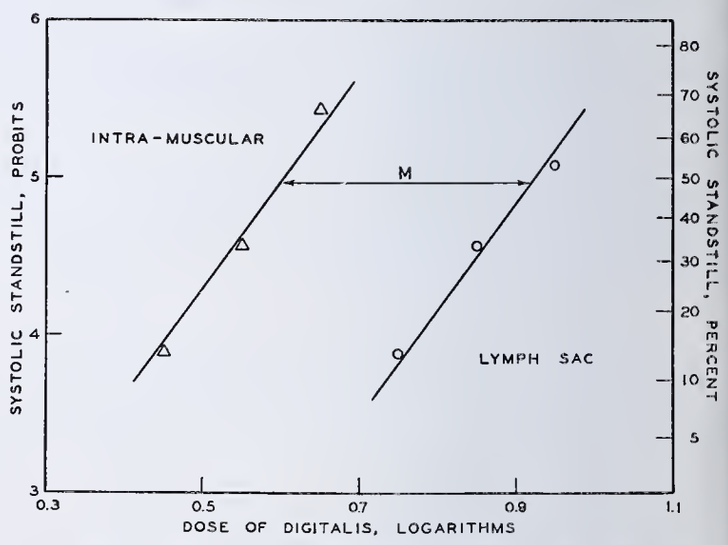

FIGURE 5. ASSAY OF EFFECT OF ROUTE OF INJECTION UPON THE POTENCY OF DIGITALIS

Data of Miller, Bliss, and Braun (*9*)

When dosages are restricted to this central section, the data can be handled by a flexible statistical procedure known as factorial analysis, which has been adapted for biological assay by Bliss and Marks (*4*). Factorial analysis not only tests whether the dosage-response curves for standard and unknown can be considered as parallel straight lines but leads directly and easily to an estimate of the log-ratio of potencies and its error, $M \pm s_M$.

Factorial arrangements have the added advantage that they are usually coupled with designs that screen out important sources of potential error, leading to unbiased estimates of drug action and marked improvements in precision. Two statistical techniques contribute to this end. The first is the balancing of known and suspected variations in susceptibility between individual animals, litters, days of the experiment, and other qualitative sources, so that they occur

equally at all dosages of the standard and the unknown. Within these restrictions the assignment of dosages to living material is strictly random. Then when the test has been completed, factors which are extraneous to the assay can be segregated from the effect of dosage and from the experimental error by the analysis of variance, usually with a marked increase in precision.

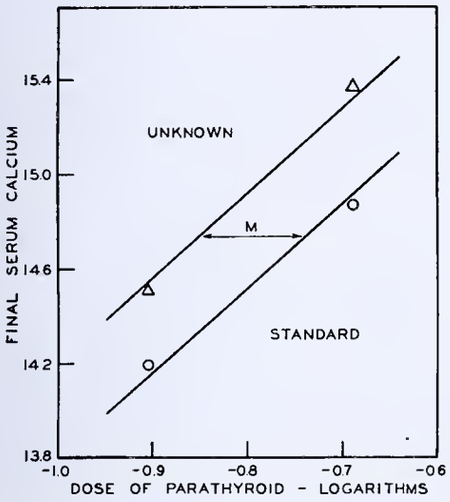

FIGURE 6. ASSAY OF AN UNKNOWN SAMPLE OF PARATHYROID EXTRACT FROM THE SERUM CALCIUM OF THE DOG
Data of Bliss and Rose (*5*)

The second technique is the use of covariance to correct graded variations in initial or concomitant measurements from the internal evidence of each assay. In the past these have been adjusted by arbitrary corrections, often involving unrecognized and doubtful assumptions which sometimes may even increase the error.

TABLE II. ANALYSIS OF VARIANCE OF A PARATHYROID ASSAY

[Arranged in five 4 × 4 Latin squares, in terms of the final calcium level 16 hours after injection; data from Bliss and Rose (*5*)]

Cause of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio
Differences between days	3	0.941	0.314	...
Differences between dogs	19	43.188	2.273	4.49
Unknown *vs.* standard	1	3.280	3.280 = D^2	6.49
Slope of dosage-response curve	1	11.705	11.705 = B^2	23.14
Departure from parallelism	1	0.162	0.162	...
Experimental error	54	27.312	0.506 = s^2	1
Total	79	86.588		

An example of an arbitrary correction is the rise in the serum calcium of the dog as a criterion for the assay of parathyroid extract. First segregating the differences between individual dogs, which can be used repeatedly, Bliss and Rose (*5*) showed by covariance that all the information needed for the assay is contained in the final measurement of serum calcium 16 to 18 hours after injecting the parathyroid extract and nothing is contributed by a knowledge of the initial level prior to treatment. Here statistical analysis has demonstrated that the number of bleedings and of chemical analyses can be cut in half without any loss of precision. Another instance is furnished by the assay of vitamin D from the bone ash of rats, where the use of the percentage ash as the criterion of response requires an unproved assumption as to the relation between the organic matter and the ash in the bone. By shifting from the percentage ash to the log-weight of ash for measuring the activity of vitamin D, Bliss (*2*) showed by covariance that the weight of bone lost in ashing contributed negligibly to the assay and that the estimate of potency was improved to the same extent as if twice as many rats had been used.

The numerical form of some of these procedures may be illustrated by the assay of parathyroid extract from the serum calcium of 20 dogs as recorded 16 hours after injection, each tested with 2 doses of standard and 2 doses of unknown in a Latin square design on 4 different days. The analysis of variance is given in Table II. To judge the relative importance of each factor in the assay, we are especially interested in the ratio of its mean square to that for error. It is evident that the dogs differed but little in their mean serum calcium on the 4 days of this particular test, but that precision was improved substantially by segregating the over-all differences between individual dogs. Since the departure from parallel dosage-response curves for standard and for unknown had a smaller mean square than the error, the assay was clearly a valid one, as shown graphically in Figure 6. The variance ratio shows that the unknown produced a significantly greater effect than the standard. Its estimated potency was computed readily from D, B, and s as $M = 0.1137 \pm 0.0505$ or 130 ± 15 per cent.

Many other applications of statistical methods to biological assay still remain and there are many possibilities yet to be explored. All the above illustrations have been drawn from experiments upon laboratory animals, but in the final analysis, no drug or assay technique can be passed until it has been checked clinically. Clinical experiments offer opportunities for improved statistical design and control fully as great as those conducted in the laboratory. In many cases relatively small changes in procedure would enable the clinician to double the reliability of his evidence or to obtain quantitative conclusions from what would otherwise constitute a roughly qualitative result. Time does not permit a further examination of this topic.

Characteristics of a Valid Biological Assay

1. Different samples of the same drug must show the same relative potencies in biological assay as under clinical test. Since the products assayed biologically are frequently complex or impure mixtures, the patient may not react to the same components as the laboratory test animal. Gold and Kwit (*8*), for example, have shown a threefold difference in the reaction of man to related cardiac glucosides judged as equipotent when tested in cats and frogs.

2. On the coordinates used for biological assay, the curve relating response to log-dose should be a straight line and relatively steep when compared with the variation about the line. Either the curve should have been shown to have a constant, known slope by repeated test over a considerable period of time or the slope should be determined as an integral part of each assay. Assumed relations between dose and effect are to be avoided.

3. The potency of the unknown or sample should be determined by comparative test with a stable reference standard and expressed in units of this standard. In a determination of potency the biological reaction has the same function as a chemical indicator and biological terms, such as the "cat unit" for digitalis, have neither the uniformity nor the correct dimensions for expressing quantity of drug.

4. The living material exposed to different doses of standard and of unknown must be as nearly equivalent as it can be made. Potential sources of variation, such as differences between individuals, litters, dates of treatment, and sexes, should never coincide or be confounded with differences in treatment but within these limitations the dosages and samples must be assigned at random. The analysis of variance or an equivalent technique should be used to segregate from the estimate of error the sources of variation that have been quarantined by the design of the assay. Variations in an initial measurement, such as the initial blood sugar in the rabbit insulin assay, or in a concomitant measurement, such as that of body weight, should be adjusted not by an assumed relation which is sometimes concealed in the definition of response but rather from the internal evidence of self-contained experiments by covariance.

5. A determination of potency should always include an estimate of its error, computed as an integral part of the assay. Not only do the assays of different drugs vary widely

in their average precision—one of those in the British Pharmacopœia (*6*) has limits of 92 to 108 per cent (Staphylococcus antitoxin), another limits of 37 to 272 per cent (vitamin A)—but the precision of the assay of a single drug varies from laboratory to laboratory and from one run to another in the same laboratory. No assay with an indeterminate error can be considered satisfactory.

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Determination of Silicon

In Monel Metal, Copper-Silicon Alloys, and Similar Nonferrous Alloys Containing Silicon

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IN THE determination of silicon in Monel metal, copper-silicon alloys, and similar nonferrous alloys containing silicon, a rapid, accurate method, to replace the longer and more commonly used methods of dehydration by nitric-sulfuric acids or hydrochloric acid and potassium chlorate, is needed for the analysis of a large number of samples. Little attention appears to have been paid to the use of perchloric acid for the dehydration of silica in these materials. Willard and Cake (*2*) proposed the use of perchloric acid as a dehydrating agent in silicon determination. Both they and Fowler (*1*) have pointed out the disadvantages of nitric, sulfuric, and hydrochloric acids in the dehydration of silica in steels, silicates, and some other metals and alloys. These same disadvantages (slow evaporation, slow solution of soluble salts, bumping and spattering of the solution, and the necessity of recovering silica from filtrates) apply to Monel metal and other nonferrous alloys containing silicon.

The following procedure, evolved by this laboratory gives accurate results in from 2 to 3 hours, eliminates the disadvantages of the nitric-sulfuric acid and hydrochloric acid procedures mentioned above, and avoids the bumping that occurs when the metal is first dissolved in nitric acid and then fumed with perchloric acid.

Procedure for Monels

Weigh 2 grams of fine drillings into a 400-ml. Pyrex beaker, add 25 ml. of 70 per cent perchloric acid and 1 ml. of concentrated nitric acid, place the beaker on a hot plate, and heat until solution of metal is complete. Add a few more drops of nitric acid if necessary to ensure complete solution. Place the beaker on an extremely hot electric or gas plate and boil the solution for about 10 minutes to ensure the complete dehydration of silica. The beaker may be heated over an open gas flame towards the end of this period and the contents of the beaker slowly revolved. Cool slightly, add water, and heat until all soluble salts are dissolved, boiling the solution at the end. Filter hot through a Whatman No. 41H or similar filter paper. Wash 5 times alternately with a solution containing a mixture of 1 per cent nitric acid and 1 per cent hydrogen peroxide, and with boiling water.

Remove filtrate for the subsequent determination of other elements and wash the silica residue with hot 10 per cent hydrochloric acid and boiling water alternately 5 times and then with water until free from acid. Place filter paper in a 30-ml. platinum crucible, ignite, cool, and weigh. Volatilize silica with hydrofluoric acid plus 3 drops of sulfuric acid, ignite again, cool, and reweigh. The loss in weight is silica (SiO_2). The residue remaining in the crucible will consist of traces of nickel, iron, and titanium. This can be fused with pyrosulfate, dissolved in water, and added to the filtrate. Some platinum will be introduced during this fusion and will be subsequently deposited with the copper and weighed as such. This has been found to be extremely small and in routine analysis can be disregarded.

The filtrate from the silicon determination can be used for the determination of copper, iron, and titanium by the following procedure:

Boil filtrate strongly to remove free chlorine, and add 1 ml. of concentrated nitric acid and 5 ml. of concentrated sulfuric acid. Keep the volume of solution at approximately 300 ml. Electroplate copper on the platinum cathode at 0.5 ampere and 3 volts. After copper is removed, make the solution weakly ammoniacal and determine iron and titanium in the usual manner. Excess ammonia will form a precipitate with perchloric acid.

Table I gives results by the three methods for determining silicon in samples of Monel metal. In each instance the filtrates were evaporated to dryness twice to recover silica. No silica was found in the filtrates from the perchloric-nitric acid treatment. In the other two cases sufficient silica was found to make these evaporations necessary.

Table I. Determination of Silicon

	Perchloric-Nitric Acid	Sulfuric-Nitric Acid	Hydrochloric Acid–Potassium Chloride
	%	%	%
Sample 1	3.02–3.03	2.99–3.01	3.07–3.10
Sample 2	1.49–1.49	1.50–1.46	1.48–1.46
Sample 3	3.08–3.09	3.04–3.19	3.08–3.12

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The views presented in this article are those of the writers and should not be construed as the official views of the Navy Department.

Determination of Ascorbic Acid in Citrus Fruit Juices

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THE chemical methods for the determination of ascorbic acid (vitamin C) have been mainly based on the method of Tillmans, Hirsch, and Hirsch (*5*) employing 2,6-dichlorophenolindophenol or on some modification of the iodometric titration. In general the iodometric methods have been subject to criticism since they lack specificity and a sharp end point, although Stevens (*3*) has shown that by employing a double back-titration in the presence of a high concentration of hydrogen ions the iodine end point may be made very sharp. Further, Tauber and Kleiner (*4*) have demonstrated that the iodometric method is adequate for the determination of ascorbic acid in citrus fruit juices, since interfering substances are absent. However, in common with all iodometric methods, the reagents employed in Stevens' method change their titer with time, requiring frequent standardization, and the double back-titration increases the volumetric error.

In the method presented here, a direct titration with iodate in acid-iodide gives an end point comparable in sharpness with that of the above double back-titration method, and in addition avoids the necessity for frequent standardization. Only one accurately prepared solution is required, and this is stable over long periods of time.

In addition to the iodate titration described below, the ascorbic acid content of the citrus juices was determined by the method of Stevens (*3*). The juices were also titrated by the Mack and Tressler (*1*) modification of the Tillmans method (*5*). The indophenol dye, recrystallized from the Eastman product, was standardized by the method of Menaker and Guerrant (*2*). All the reagents used in the three methods were referred to a single primary standard solution, 0.1 *N* potassium iodate.

REAGENTS. Standard iodate, 0.1 *N* (0.0167 *M*), contains 3.567 grams of potassium iodate per liter. For use this stock solution is diluted to 0.01 *N*.

Potassium iodide, 10 per cent solution fresh daily.

Sulfuric acid, 2 *N*.

Starch indicator is made fresh daily from improved Lintner's soluble starch, using 1 gram per 100 ml. with 2 grams of potassium iodide added.

PROCEDURE. Add to 5 ml. of citrus fruit juice 1 ml. of 10 per cent potassium iodide and 2 ml. of 2 *N* sulfuric acid. Titrate the resulting solution with 0.01 *N* iodate, adding the reagent dropwise near the end point. It is best not to add the starch until very near the end point.

1 ml. of 0.01 *N* iodate = 0.88 mg. of ascorbic acid.

Results and Discussion

A preliminary comparison of the three methods employed for the determination of ascorbic acid was carried out on solutions of synthetic, crystalline ascorbic acid (Merck). The results, presented in Table I, show that the indophenol and the iodate titration are in agreement, although the absolute amounts recovered were slightly high. On the other hand, the double back-titration of Stevens gave slightly low values for the ascorbic acid content.

Analyses on citrus juices are recorded in Table II. The results obtained by the Stevens and iodate methods are compared with the ascorbic acid content as determined by the Mack and Tressler indophenol titration as a standard. Stevens' method gives variable results that differ rather widely from those determined by the indophenol method, being up to 12 per cent too high. On the other hand, the ascorbic acid content as determined by the iodate method agrees with that found by the indophenol titration. On the basis of these results the iodate method can be recommended for routine determinations of ascorbic acid in citrus juices.

The iodate titration has the following advantages over the double back-titration method of Stevens: increased accuracy, stability of reagents, greater simplicity and rapidity, and more reproducible results.

TABLE I. DETERMINATION OF ASCORBIC ACID

Ascorbic Acid Taken	Indophenol Titration Ascorbic acid found	Recovery	Stevens' Iodine Titration Ascorbic acid found	Recovery	Iodate Titration Ascorbic acid found	Recovery
Mg.	*Mg.*	%	*Mg.*	%	*Mg.*	%
1.75	1.81	103.4	1.80	102.8	1.77	101.7
2.00	2.09	104.5	1.88	94.0	2.08	103.9
2.00	2.09	104.5	1.90	95.0	2.07	103.6
3.00	3.10	103.3	2.96	98.6	2.97	99.0

TABLE II. ANALYSES OF CITRUS JUICES

Material	Indophenol Titration	Stevens' Iodine Titration	Difference from indophenol titration	Iodate Titration	Difference from indophenol titration	Difference from Stevens' iodine titration
	G./100 ml.	*G./100 ml.*	%	*G./100 ml.*	%	%
Lemon juice, 1	0.0460	0.0470		0.0460		
		0.0471	2.1	0.0461	0.0	−2.1
Lemon juice, 2	0.0427	0.0465		0.0434		
		0.0461	8.4	0.0438	2.1	−6.2
Lemon juice, 3	0.0462	0.0460	− 0.5	0.0462	0.0	0.5
Lemon juice, 4	0.0396	0.0397	0.2	0.0392	−1.0	−1.2
Lemon juice, 5	0.0416	0.0458				
		0.0448	8.8	0.0424	1.9	−6.4
Orange juice, 1	0.0416	0.0420		0.0419		
		0.0420	0.9	0.0417	0.5	−0.4
Orange juice, 2	0.0388	0.0390		0.0387		
		0.0388	0.2	0.0387	−0.3	−0.5
Orange juice, 3	0.0399	0.0346		0.0342		
		0.0344	1.7	0.0341	0.6	−1.1
Grapefruit juice, 1	0.0424	0.0461		0.0446		
	0.0430	0.0470	9.8	0.0442	4.0	−4.5
Grapefruit juice, 2	0.0370	0.0412		0.0372		
	0.0370	0.0416	11.8	0.0378	1.4	−9.4

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Determination of Bromine Addition Number

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A method is described for indicating degree of unsaturation quickly, by direct titration of sample dissolved in chloroform with a standard solution of bromine in glacial acetic acid, using the color of the bromine itself as indicator. It has given correct results when applied to pure hydrocarbons of known unsaturation, such as cyclohexene, diisobutene, triisobutene, etc., and reproducible results on unknowns. It was found that the solvents used in the analysis are factors affecting the results.

The method may also find utility in fat analysis.

SINCE it is practically impossible to separate the numerous components of complex hydrocarbon mixtures, such as naphtha and gasoline, one must still be content with an analysis that indicates the proportions of classes of hydrocarbons. Methods have been published for analytically resolving the components of gasoline into four major groups—unsaturates, aromatics, naphthenes, and paraffins. A good degree of accuracy is frequently claimed for such methods of analysis when the latter three classes comprise the gasoline; much less is claimed when unsaturated hydrocarbons are also present.

Modern processes for manufacturing gasoline have made unsaturated hydrocarbons more important than ever before, and accurate knowledge of degree of unsaturation an urgent requirement.

The many published methods for determining unsaturation of oils, etc., fall into two major groups:

1. Those in which the unsaturates are dissolved or chemically transformed and separated, such as methods involving the use of mercuric acetate (*15*), sulfur chloride (*2*), and sulfuric acid (*17*).
2. Methods based on reactions with halogen, in which unsaturates are not separated but merely reacted for titrations.

None of the methods in group 1 is entirely satisfactory. Brame and Hunter (*1*) found the mercuric acetate (*15*) method unreliable because different unsaturated hydrocarbons react differently with the reagent. The sulfur chloride method (*2*) is rather involved for routine use, and procedures which employ sulfuric acid (*17*) are particularly unsuited for modern cracked gasoline because of alkylation reactions which sulfuric acid may induce (*7*), causing aromatics and isoparaffins, at least, to appear in the analytical result for olefins.

Most methods of group 2 were developed for the analysis of saponifiable fats and oils and their application to hydrocarbons meets with very limited success, the magnitude of the result being influenced to a large extent by the excess of reagent, as can be seen from Figures 1 and 2. The Hubl, Hanus, and Wijs procedures are well-known examples of such methods. Many attempts have been made to devise more reliable methods for determining unsaturation. Margosches (*12*) allows an ethyl alcoholic iodine solution to react with hydrocarbons in presence of water. However, other investigators (*9*) report that this method gives values that are too low. Grosse-Oetringhaus (*5*) reviewed and investigated numerous methods and concluded that Kaufmann's method (*8*) is reliable. This method was investigated by the present authors and is discussed below. Kaufmann employs an excess of bromine solution in methyl alcohol saturated with sodium bromide and makes no correction for substitution.

The bromine method of McIlhiney (*11*) gained prominence, apparently because it provided means for distinguishing bromine consumed for addition reactions from that for substitution, the substituted bromine being calculated from the resultant hydrobromic acid. Evidence has been accumulating, however, which discredits the general suitability of this procedure. Pure hydrocarbons of known unsaturation have yielded results for bromine addition number by the McIlhiney method which were not only too low but in numerous instances were negative values, indicating that reactions other than addition and substitution occurred during the analysis. Grosse-Oetringhaus (*5*) concludes that the McIlhiney method is of value only in those cases where substitution has not occurred as indicated by the absence of hydrobromic acid in the reaction products. The same limitation appears to apply to the other methods mentioned—a condition probably commonly experienced with saponifiable fats, but rarely with hydrocarbon mixtures.

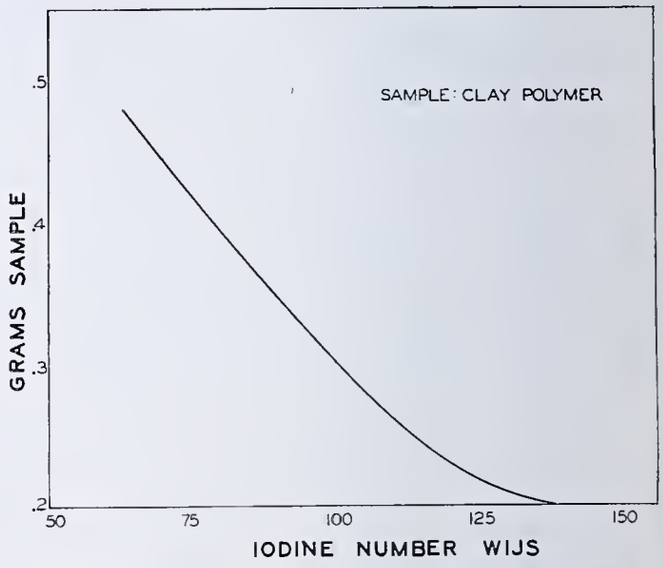

FIGURE 1. EFFECT OF SAMPLE SIZE ON MAGNITUDE OF IODINE NUMBER

Amount of reagent constant and in every case so large that at least half was left unconsumed at end of test.

Most methods for determining unsaturation employ an excess of the halogen, apparently to ensure complete halogenation, the excess being titrated after the reaction period. Francis (*3*) seeks to avoid substitution by keeping the excess of bromine low through controlled addition of sulfuric acid to his potassium bromide-bromate reagent which upon acidification liberates bromine.

To study the effect of excessive bromine on bromine addition number, triisobutene was tested both by Francis' and Kaufmann's methods, using various excesses. The results of these tests are shown in Table I. Triisobutene was chosen because its bromine addition number could not be deter-

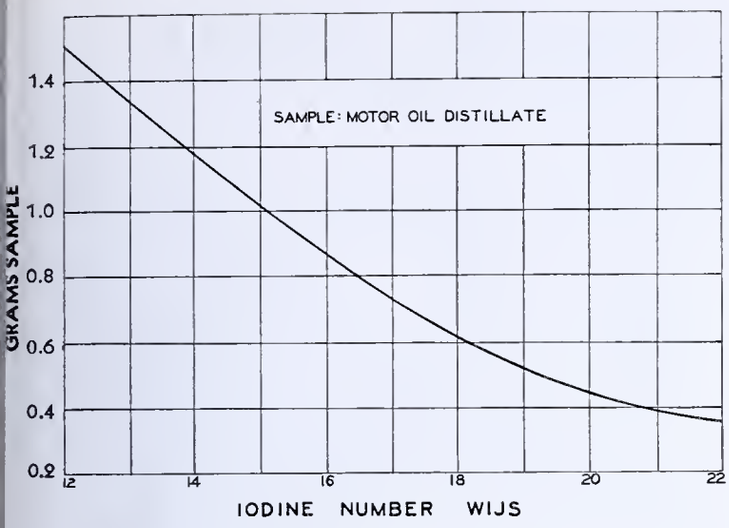

FIGURE 2. EFFECT OF SAMPLE SIZE ON MAGNITUDE OF IODINE NUMBER

Amount of reagent constant and in every case so large that at least half was left unconsumed at end of test.

mined simply and accurately by methods previously employed by the authors.

These results clearly indicate a relationship between excess of bromine and magnitude of bromine number. Though theoretical values are obtainable by such methods, they suffer the disadvantage of requiring a series of trial titrations to determine the optimum excess of bromine.

Thomas, Bloch, and Hoekstra (*16*) have improved the Francis method by cooling in many steps. However, the present authors have found even this improved procedure to yield low results for triisobutene (58 to 62 *vs.* theoretical 95) and tetraisobutene (45 to 50 *vs.* theoretical 71). These low values have been confirmed by Lewis and Bradstreet (*10*) in their further improvement of the Francis method, which was published after the present paper had been submitted for presentation at the September, 1940, meeting of the AMERICAN CHEMICAL SOCIETY.

The data shown in Table I caused the authors to investigate the feasibility of titrating with bromine, since by such means the bromine would never be in excess. Morrell and Levine (*13*) described a method based on this principle, titrating in diffused light with bromine solution (4 per cent by volume in carbon tetrachloride) which has been standardized against a pure olefin, olefin-free naphtha being recommended as solvent for the sample.

Normann (*14*) and Hofmann (*6*) find the nature of the solvent to be an important factor in bromination reactions, and the present authors find that carbon tetrachloride tends to retard the reaction and frequently renders the end point uncertain. Chloroform as solvent for bromine and sample was not consistently satisfactory; nor was olefin-free naphtha (A. S. T. M. precipitation naphtha and Kahlbaum's normal benzine) an improvement when employed as sample solvent. The bromine addition number of triisobutene, for example, could not be obtained with naphtha as sample solvent, though it was easily determined with chloroform as sample solvent, the titration in each case being made with a solution of bromine in glacial acetic acid, using bromine color for end point. The chloroform–glacial acetic acid solvent combination proved very satisfactory, end points being sharp and absorption of bromine rapid.

The question of whether or not substitution occurs in the proposed method was not directly investigated because of the intricacy of the problem. However, since Kaufmann's and Francis' methods give bromine numbers which approach theoretical addition values as the excess of bromine employed becomes smaller, and since the proposed method employs no excess of bromine and yields theoretical values, it seems a justified conclusion that substitution does not occur.

The following are the details of the method adopted by the authors.

Apparatus and Reagents

A 10-ml. buret divided in 0.05 ml., a 50-ml. buret divided in 0.1 ml., 25-ml. Erlenmeyer flasks, a 5-ml. pipet, a 1-ml. pipet divided in 0.1 ml., and 250-ml. glass-stoppered iodometric flasks.

Bromine (2 per cent by volume) in C. P. glacial acetic acid (keep in a dark, glass-stoppered bottle), 0.1 *N* sodium thiosulfate, C. P. chloroform, 10 per cent potassium iodide solution, and starch solution.

TABLE I. INFLUENCE OF EXCESS BROMINE ON APPARENT BROMINE ADDITION NUMBER

(Hydrocarbon, triisobutene)

Size of Sample	Excess of Bromine	Bromine Addition Number Found	Bromine Addition Number Theoretical
Grams	*Mg.*		
	Francis' Method		
0.382	121	130	
0.382	93	127	
0.382	70	119	
0.382	64	113	95
0.382	54	103	
0.382	8	97	
0.382	4	87	
0.382	2	77	
	Kaufmann's Method		
0.151	215	120	
0.151	144	117	
0.151	77	110	95
0.151	10	99	
0.151	0	94	

Procedure

Weigh 0.100 to 1.000 gram of sample, depending on the degree of unsaturation, into a 250-ml. Erlenmeyer flask and to it add 5 ml. of chloroform. Volatile samples are best measured (0.1 to 1.0 ml.) into 5 ml. of chloroform, and the weight of the sample calculated from its gravity. Titrate the sample directly (no indicator) with bromine reagent, adding it until a distinct orange-yellow color is obtained which persists for about 15 seconds. With a little experience it is possible to add the bromine solution rapidly at first and in 0.05-ml. portions toward the end of the titration.

The end point is obscure with some dark samples. In these cases, add the bromine reagent slowly, especially when approaching the end point, and add with a glass rod one drop of the chloroform solution of the sample to about 1 ml. of potassium iodide–starch solution kept in the cavity of a spot plate. The appearance of the blue color marks the end point. This latter procedure is recommended only for dark samples with which the end point cannot otherwise be ascertained.

Determine the titer of the bromine reagent by introducing 5 ml. of the bromine solution into 25 ml. of 10 per cent potassium iodide solution and 5 ml. of C. P. chloroform in a 250-ml. iodometric flask, and titrating promptly with 0.1 *N* thiosulfate in the usual manner, using starch as indicator toward the end of the titration.

CALCULATIONS:

1 ml. of 0.1 *N* thiosulfate $\simeq$ 7.992 mg. of bromine

$$\frac{\text{Mg. of Br per ml. of Br reagent} \times \text{ml. of Br reagent consumed} \times 100}{\text{wt. of sample in mg.}} = \text{bromine addition number}$$

Precision and Accuracy

The precision and accuracy of the proposed method, applied in a routine manner, are evidenced by Table II.

Some samples of diisobutene have been encountered where reproducibility has not been too good—for example, 10 de-

Table II. Bromine Addition Numbers

Actual Composition of Samples: Kahlbaum normal benzine	Cyclohexene	Diisobutene	Triisobutene	Bromine Number Found	Addition Number Theoretical
100	...	...	...	0	0
100	...	...	...	0	
...	100	...	...	198	194
...	100	...	...	193	
...	...	100	...	140	143
...	...	100	...	144	
...	...	...	100	93	95
...	...	...	100	93	
50	50	...	...	100	97
50	50	...	...	100	
50	...	50	...	71	72
50	...	50	...	71	
50	...	...	50	43	
50	...	...	50	45	
50	...	...	50	46	47
50	...	...	50	46	
50	...	...	50	46	
50	25	25	...	80	84
50	25	25	...	83	
50	...	25	25	61	59
50	...	25	25	57	
25	25	25	25	104	107
25	25	25	25	106	
Styrene				153	154
Benzene, c. p.				0	0
Toluene, commercial				2–2	0
Xylenes, commercial				3–3	0
Triisopropylbenzene, commercial				1–1	0
Oleic acid, c. p.				58	57
Cyclohexane, c. p.				0–0	0
Isopentane, c. p.				0–0	0
Tetraisobutene				69–72	71

terminations on a sample varied from 131 to 142 *vs.* a theoretical bromine addition number of 143. It was found, however, that if one drop of water was added to the contents of the flask just before commencing the titration, excellent reproducibility and improved accuracy were obtained, the same sample run 10 times yielding results of 139 to 142. The recommendation to add a drop of water is made for this hydrocarbon only, as it is generally unnecessary and may prove detrimental with some.

The results presented in Table II indicate that although the method is primarily intended for hydrocarbons of the gasoline range, it can successfully be applied to other compounds, such as fatty acids and aromatic compounds having an olefin linkage; in the latter the aromatic double bond is not affected.

The possible interference of such substances as sulfur dioxide, hydrogen sulfide, acids, or mercaptans, all of which may conceivably be present in naphthas at some stage of their processing, can be eliminated by treating the naphtha with an alkaline lead solution (doctor solution) and filtering prior to making the bromine number test.

The proposed method was found also to give reproducible and correct values on finished gasolines, neither the dye nor the lead (3 cc. of tetraethyllead per gallon, 3.785 liters) interfering.

Table III. Comparative Bromine Addition Numbers by Direct Titration and McIlhiney Methods on Unknowns

Sample No.	Bromine Addition Number Direct titration	McIlhiney method
1	95	39
2	140	−5
3	151	24
4	0	0
5	16	5

Other Applications

The authors feel that it may be possible to advance the proposed method as a rapid substitute for the usual procedure for iodine value, as it requires only a few minutes, compared with over an hour. It might thus become a useful control method to follow hydrogenation or oxidation (blowing) processes.

In this connection a few experiments were made to compare the indications of unsaturation of fats by the new method with that by the Wijs method. The results are shown in Table IV.

In the cases of tallow, castor oil, and blown rapeseed oil, results by the two methods generally agree, while in others the difference is large. Hydrogen numbers reported in the literature (*4*) were calculated to bromine and compared with the results of the other two methods; however, hydrogen numbers were found for only two of the fats tested. The hydrogen number of linseed oil (≃ Br 78), which showed the biggest difference for results obtained by the Wijs and direct titration methods, checks that obtained by the latter. The higher results obtained by the Wijs method may be due to substitution, since the reagent is used in excess. The divergence in the case of castor oil (hydrogen number ≃ Br 50) is unexplained at this writing. Saturation by bromine of only one double bond of fats containing conjugated double bonds appears a partial explanation, confirmed by the values obtained for tung oil whose acids have three conjugated double bonds with a theoretical bromine addition number of 172.

The experiments on fats were not exhaustive but are believed sufficient to indicate utility for the proposed method in the fatty oil industries.

Table IV. Comparative Unsaturation of Fats by Wijs and Direct Titration Methods

Nature of Fat	Unsaturation by Wijs Method As % I_2	Unsaturation by Wijs Method As % Br_2	Unsaturation by Direct Titration Method As % I_2	Unsaturation by Direct Titration Method As % Br_2
Tallow	42	26	44	28
Cottonseed oil	112	71	84	53
Castor oil	90	57	88	55
Lard oil	75	47	67	42
Linseed oil	182	115	123	79
Rapeseed oil	105	66	88	55
Blown rapeseed oil 1	63	40	62	39
Blown rapeseed oil 2	59	37	61	38
Blown rapeseed oil 3	62	39	53	33
Tung oil	...	...	89	56

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Aliquant Samples in the Determination of Fluorides in Mixed Foods

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IN A STUDY of the fluoride content of the normal diet it became necessary to ash total 24-hour mixed food samples, including solids, semisolids, and liquids that were not true solutions. (Fluids that were true solutions were collected and analyzed separately.) Ashing the sample in its entirety was difficult, fats in the foods causing particular difficulty, and the time required for drying and ashing the entire sample was found to be prohibitive. Consequently an attempt was made to devise a method of macerating the sample, mixing it to homogeneity, and aliquanting.

Since acid digestion could not be used, mechanical maceration was tried. Grinding and thorough mixing with an electric food mixer produced such apparent homogeneity as to justify the attempt to use aliquants.

A series of 27 samples ranging from 900 to 1500 grams and representing the food intake of an experimental subject on 27 successive days, was ground and mixed, and 300-gram aliquants were removed, both aliquants and remainders being ashed and analyzed. The 300-gram sample was usually completely ashed and ready for analysis after 6 to 12 hours in the muffle furnace. In the case of the large remainder, 4 to 6 times as much time was required for drying and ashing.

In Table I the mean value of the results on the aliquants is compared with that on the remainders. The mean value of the results obtained from the aliquants did not differ significantly from that from the remainders. Especially significant is the greater coefficient of variability for fluoride values obtained from the remainders. As the latter were bulky, the greater variability of these results is attributed to the difficulties inherent in handling and ashing such large samples.

Since the 4.5 to 7.0 grams of ash (and magnesium oxide) from 300 grams of food were greater than the amount necessary for distillation, an attempt was made to reduce the size of the aliquant. Food samples for 25 successive days were used to compare 150-gram aliquants with 300-gram quantities. Comparison of the mean value of results obtained by analysis of the smaller aliquants with that of the large aliquants is given in Table II.

TABLE I. COMPARATIVE RESULTS

	No. of Samples	Mean Fluoride Content	Standard Deviation	Coefficient of Variability
		Micrograms	*Micrograms*	
300-gram aliquants	27	248 ±9.2	±70	28
Remainders after removal of 300-gram aliquants	27	249 ±13.1	±101	40

No significant differences exist between the mean values for the results obtained with the two sizes of aliquants. The means are stable and there is no significant difference between their coefficients of variability. Therefore, the use of aliquants of 150 grams or more will yield satisfactory results.

The second series of samples was obtained some time after the first. Variation in the diet probably explains the lower mean fluoride content of the second series.

TABLE II. COMPARATIVE RESULTS

(Remainders not analyzed)

	No. of Samples	Mean Fluoride Content	Standard Deviation	Coefficient of Variability
		Micrograms	*Micrograms*	
300-gram aliquants	25	158 ±6.2	±46	29
150-gram aliquants	25	161 ±6.8	±50	31

FIGURE 1. FOOD MIXER

Method

The 24-hour food sample after collection in glass jars is ground in a household electric mixer with grinding attachment (Figure 1). The wet weight is determined and magnesium peroxide is added at the rate of 1.0 gram per 100 grams of wet food. (Magnesium peroxide having the lowest fluoride blank obtainable should be procured.) The magnesium peroxide is mixed with the food for from 0.5 to 1.5 hours, depending upon the time required to obtain a homogeneous mixture. Two 150-gram aliquants are weighed into 200-ml. nickel evaporating dishes and dried on an electric hot plate. The fats are driven out by charring on an electric burner and the sample is then placed in the automatically controlled electric muffle furnace, set at 570° C. After complete ashing, the ash is weighed and prepared for distillation.

The fluoride is separated from the ash by distillation with perchloric acid according to MacIntire and Hammond's (*3*) modification of the method of Willard and Winter (*4*). Silver sulfate, as recommended by McClure (*2*), is added to the ash to prevent volatilization of the chlorides.

The back-titration method of Dahle, Bonnar, and Wichman (*1*) is employed from this point on; the unconcentrated distillate is used.

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Determination of Iron by the Zimmerman-Reinhardt Method

Effects of Temperature and Determination of Blank

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IN THE various procedures for determination of iron by the Zimmerman-Reinhardt method as outlined in standard texts on analytical chemistry, there seems to be a fairly general impression that the treatment with mercuric chloride and the titration with permanganate are favored by having the solutions at a low rather than a high temperature, and that the determination of the blank does not require the presence of ferric chloride in the solution, although it is one of the end-point products.

Regarding the mercuric chloride treatment one reads such statements as "cool at least to room temperature", "cool completely", "the temperature should not be over 25° C.", etc. Concerning the permanganate titration the instructions vary from "titrate the cool solution" to "dilute with ice-cold distilled water". In the matter of the blank the following quotation is typical: "A blank should be carried out on the reagents used in the analysis." In the many articles dealing with the Zimmerman-Reinhardt method evidently the temperature effects were not considered worthy of special consideration or comment, and there appears to be no detailed information as to the proper method of obtaining a blank correction (*1–5, 7–10*). It is of significance to note that Meineke has shown that it is possible for the ferric chloride to be reduced by the mercurous chloride and that the extent of the reaction depends upon the amount of mercurous chloride present (*6*).

It has been the experience of the present authors that within the range of 10° to 40° C. the results are little affected by the temperature during the mercuric chloride treatment, but that during the permanganate titration there is a noticeable advantage in having the temperature around 30° rather than 10° C. In work of approximately 0.1 per cent accuracy the ferric chloride has an effect on the value of the blank by no means negligible. Tables I and II are offered in support of these statements.

TABLE I. DEVIATIONS OF ZIMMERMAN-REINHARDT FROM JONES REDUCTOR METHOD

(At different temperatures of addition of mercuric chloride and permanganate)

Temperature of $HgCl_2$ Addition, ° C.	Temperature of Titration				
	10° C.	20° C.	25° C.	30° C.	40° C.
	Ml. of 0.1122 *N* $KMnO_4$				
10	0.05	0.05	0.04	0.04	0.05
20	0.04	0.05	0.05	0.05	0.05
25	0.05	0.04	0.04	0.04	0.05
30	0.04	0.03	0.03	0.03	0.06
40	0.04	0.03	0.03	0.04	0.06

Table I shows the effects of the temperature of the solutions during the mercurous chloride precipitation and the titration with permanganate within the temperature limits of 10° to 40° C. The deviations are the number of milliliters of 0.1122 *N* permanganate by which the Zimmerman-Reinhardt method exceeds the Jones reductor method in the titration of 25.00 ml. of approximately 0.17 *N* ferric chloride solution. Under the conditions of the experiments the results are apparently little affected by the temperature of the mixture during the mercuric chloride treatment, since the deviations at 10° C. are so little different from those at 40° C. If there is any advantage at all it is probably at the higher temperature. As for the titration temperature, the deviations do not indicate that there is any advantage in cooling the solution to 10°, nor does there appear to be any harm in having the temperature as high as 30° C. In the actual titrations, however, the end points at 30° and 40° were somewhat sharper and did not fade so rapidly as at the lower temperatures. It is probably inadvisable to have the titration temperature as high as 40°, as column 6 begins to show a definite trend toward higher results. In Tables I and II all values are the results of 3 to 5 determinations whose average deviations were not greater than 0.01 ml.

TABLE II. DETERMINATION OF BLANK

Experiment	Temp. ° C.	Total Volume of Solution Ml.	Volume of $SnCl_4$ Solution Ml.	Volume of $FeCl_3$ Solution Ml.	Volume of Preventive Solution Ml.	Volume of 0.1022 *N* $KMnO_4$ Required Ml.
1	10	500	10	20	25	0.16
2	20	500	10	20	25	0.16
3	40	500	10	20	25	0.16
4	26	500	5	10	25	0.12
5	26	300	5	10	25	0.10
6	26	100	5	10	25	0.09
7	26	500	5	0	25	0.10
8	26	500	5	5	25	0.11
9	26	500	5	20	25	0.16
10	26	500	5	10	10	0.14
11	26	500	5	10	40	0.12

Table II shows the effects of temperature, total volume of solution, and amounts of stannic chloride, preventive solution, and ferric chloride on the size of the blank for the amounts of mercurous chloride, mercuric chloride, and hydrochloric acid that might be present in a typical analysis. The values of the blank are expressed in milliliters of 0.1022 *N* permanganate. The results indicate that under the procedure used the value of the blank is not affected by temperature (experiments 1, 2, and 3) nor by the amount of stannic chloride (experiments 3 and 9), but that it does depend upon the volume of the solution (experiments 4, 5, and 6), the amount of preventive solution until a certain limit is reached (experiments 4, 10, and 11), and the amount of ferric chloride (experiments 4, 7, 8, and 9). The important point to observe is the definite effect of the ferric chloride.

The fact that the Zimmerman-Reinhardt method is slightly higher than the Jones reductor method, even under optimum conditions (0.08 per cent), may be due to an inherent difference between the two methods or to personal factors. Mixer and Dubois have reported results obtained by the two methods in which the Zimmerman-Reinhardt method was slightly lower (0.07 per cent, *6*). Barneby's work indicates that this method shows no appreciable error (*1*), while results of Jones and Jeffrey (*4*), Harrison and Perkin (*2*), and Skrabal (*9*) show trends that are distinctly high.

Reagents

Preventive solution: 67 grams of manganous sulfate ($MnSO_4 \cdot 4H_2O$), 175 ml. of phosphoric acid (sp. gr. 1.7), and 133 ml. of concentrated sulfuric acid in 1 liter of solution. Stannous chloride: 50 grams of the iron-free dihydrate to 1 liter of solution 2 *N* in hydrochloric acid. Mercuric chloride: a saturated solution containing 5 ml. of 6 *N* hydrochloric acid in each liter. Ferric chloride: a 0.1695 *N* solution 3 *N* in hydrochloric acid. Potassium permanganate: 0.1122 *N* and 0.1022 *N* solutions.

Procedure

To standardize the ferric chloride solution by the Jones reductor method, 25.00 ml. of solution were fumed with 10 ml. of concentrated sulfuric acid, 150 ml. of water were added, and the ferric sulfate was dissolved, cooled to room temperature, and rinsed through a Jones reductor until the final volume was about 400 ml. Titration with permanganate followed, the end point taken being the appearance of faint pink which persisted for at least 30 seconds.

The blank was determined as follows: First, the blank of the reductor was determined from the difference between the permanganate titers at room temperature of two 400-ml. solutions containing 10 ml. of concentrated sulfuric acid, one of which had been passed through the reductor and the other had not. The total blank for the process was obtained by adding this blank to that obtained on 400 ml. of solution containing 25 ml. of the iron solution which had been fumed with 10 ml. of concentrated sulfuric acid. Titrations of reduced iron solutions and blank determinations made with the addition of 25 ml. of preventive solution produced the same results but yielded more satisfactory end points.

In the procedure for the Zimmerman-Reinhardt method, which closely conformed to that of Barneby (*1*), 25.00 ml. of the ferric chloride solution were heated to 90° C., and stannous chloride solution was added until the yellow color disappeared, followed by 2 drops in excess. The solution was adjusted to the desired temperature and 10 ml. of mercuric chloride at the same temperature were added with thorough mixing. After about 2 minutes the resulting solution was washed into a beaker containing about 400 ml. of water and 25 ml. of preventive solution. The solution was adjusted to the desired temperature and with thorough stirring titrated with permanganate at an average rate of about 3 seconds per ml. but more slowly toward the end, until a faint pink was obtained which persisted for 15 to 30 seconds.

The blank was determined as follows: The same volume of stannous chloride solution as that used in reducing the iron (approximately 10 ml.) was oxidized with permanganate until the solution was faintly pink, and 2 drops of stannous chloride solution were added, followed by 10 ml. of mercuric chloride solution and, after 2 minutes, 420 ml. of water containing 25 ml. of preventive solution. Finally 25 ml. of the ferric chloride solution were introduced, and the solution was allowed to stand 2 minutes (the approximate duration of the titration) and titrated with permanganate as in the regular determinations. With the exception of the conditions indicated, the blanks in Table II were obtained by essentially the same procedure.

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Distillation of Foaming Solutions under Vacuum

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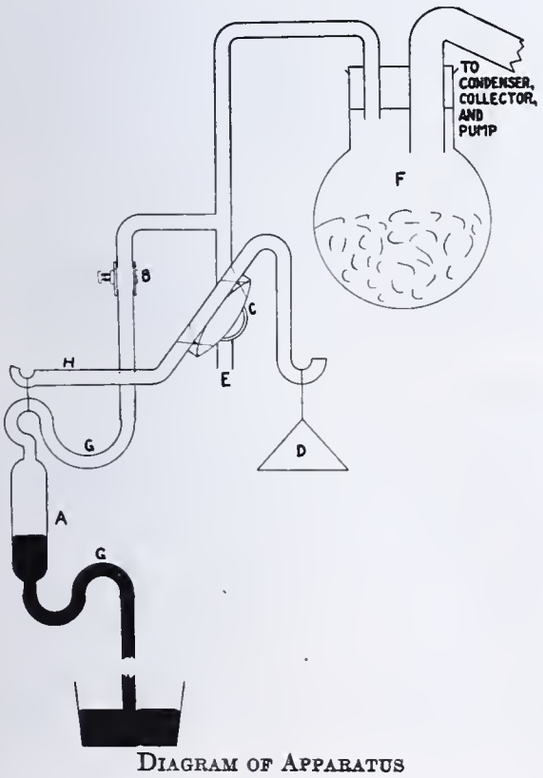

DIAGRAM OF APPARATUS

A. Glass bulb connected with rubber tubes, *G*, and supported by lever arm, *H*, by means of wire connected at gooseneck
B. Rubber connection and pinchcock
C. Glass stopcock with lever arm, *H*, attached by wire binding. Between *H* and the stopcock handle are two small rubber blocks, one at each end of the handle, making the attachment less rigid and less liable to break
D. Counterbalance attached by wire to *H*
E. Air intake
F. Distillation flask
G. Easily flexible rubber tubing (vacuum)
H. Glass lever arm (bent from glass rod)

THIS device for the prevention of excessive foaming operates on the principle that, in the distillation of foaming solutions under vacuum, a slight decrease in vacuum temporarily arrests bubble formation and destroys built-up foam masses.

The height of bulb *A* in respect to the mercury dish is adjusted so that as the vacuum approaches the point of bubble formation, the mercury will have been drawn up into *A*. The weight of the counterbalance, *D*, is such that when *A* is full, or nearly so, the total weight of the mercury, *A*, and attached tubing, *G*, overbalances *D* and pulls the lever arm, *H*, down, opening stopcock *C*, and allowing a small amount of air to enter the system through opening *E*. The vacuum of the system is thereby lowered, allowing the mercury to fall in its column out of *A*. The loss in weight allows *D* to raise the empty bulb, *A*, and reclose *C*. The pump, operating continuously, then raises the vacuum again and the process is repeated.

The pinchcock, *B*, aids in regulating the frequency of the operation by partly closing off the *A* portion of the system from the main vacuum system of which the distillation flask, *F*, is a member. The partial closure of *B* makes a lowering of the vacuum in distillation flask, *F*, system less quickly felt in the *A* system, so that, once down, the bulb tends to remain down longer than if the connection at *B* were left clear. If desired, a similar pinchcock (not shown) may be placed in the system between *C* and *F* above the side connection leading to *A*. In this case, an opening of *C* produces more quickly a drop in vacuum in the *A* system, allowing the mercury to fall faster out of *A*, thus raising the frequency of the operation but allowing less air to enter the system per phase.

The final bend in lever arm, *H*, at the point supporting *A* should not be made until the optimum point for the particular apparatus is found. If the arm from *C* toward *A* is too long, a lowering of that end of the lower arm will produce an undue lowering of the height of the mercury column in respect to the mercury dish and the bulb will remain full until the vacuum of the system, measured in height of mercury, has fallen to a point equal to the new height of the mercury column. On the other hand, if the arm is too short, there will be no appreciable lowering of the mercury column and the bulb will seek a point of equilibrium in which *C* will remain partially open and a constant vacuum will be pro-

duced. The length of *H* from the central axis of *C* to the point of attachment to *A* in the apparatus is about 14 cm.

The degree of vacuum may be regulated by adjusting the height of the mercury column—i. e., raising or lowering the mercury dish and/or decreasing the weight, *D*.

With the apparatus described, evacuated to 12 mm. of mercury with a glass water pump, it was possible to produce a fluctuation of vacuum ranging from 5 mm. to several centimeters. The same fluctuations could be produced as well at lower vacuums. The frequency of the operation could be adjusted from 8 to 20 phases per minute.

Inasmuch as a lowering of vacuum raises the boiling point of a substance, fluctuation of vacuum in this apparatus will produce a corresponding fluctuation in the boiling point of the solvent. The same precaution should be taken with this apparatus as with a manually controlled apparatus—i. e., heat should be applied to the distillation flask in such a way that, with the decreases in vacuum, the temperature does not rise above the decomposition point of the material being treated. With this apparatus a water bath was used, the temperature of which was maintained at a point below the decomposition temperature of the most heat-sensitive substance in the solution being distilled.

If it is desired to carry on the distillation in the presence of an inert atmosphere (carbon dioxide was used in this case), appropriate connections may be made from the gas cylinder or generator to *E*.

If desired, capillary tubes may be used in *F*. With badly foaming solutions, little advantage was realized, the tubes tending to "blow up" bubbles formed. If an inert gas is used, gas connections must also be made to the capillary tubes.

The apparatus has been successfully used in the distillation of water and 50 per cent water–alcohol extracts of animal tissues. The speed of the distillation is approximately the same as for a carefully controlled manual operation.

New Color Reactions for *Cannabis sativa* Resin

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THE recognition of the resinous constituent of *Cannabis sativa* (*Cannabis indica*) has been rendered difficult by the absence of reliable and specific chemical tests.

A clue to the nature of suspected material can often be obtained by microscopic examination; the odor developed by heating the residue of a petroleum ether extract at the temperature of the water bath is also of diagnostic value, provided the residue is free from lipoid and other materials extracted with the resin during toxicological investigations.

Beam (*1*) developed a delicate test, using alcoholic potassium hydroxide, and for many years this test has been of distinct service to the analyst in the East. Doubt has been expressed in regard to its specificity, and moreover it has failed on occasions with genuine plant material and pharmacopoeia preparations.

Recently, several new color reactions have been developed, notably those of Munch (*4*) and Ghamrawy (*3*) using *p*-dimethylaminobenzaldehyde and (*2*) Duquénois and Moustapha using vanillin and acetaldehyde in the presence of ethyl alcohol.

In the Duquénois and Moustapha method the colors are developed in the presence of hydrochloric acid, and in the technique of Ghamrawy and of Munch sulfuric acid is used.

In this laboratory, a considerable number of samples of ganja, charas, and bhang come under observation and it was considered that an examination of these new methods would be of distinct service in connection with forensic chemical work.

The reactions of Ghamrawy, Duquénois and Moustapha, and Munch were given by fifteen samples of *Cannabis sativa* resin examined in this laboratory and appear to be delicate tests for the resin; definite color reactions are also obtained with benzaldehyde, *o*-nitrobenzaldehyde, and salicylaldehyde; the color is best developed with hydrochloric acid; the reaction is not obtained with aliphatic aldehydes and therefore appears to be specific for aromatic aldehydes of the type studied; the reaction can be carried out in the presence of solvents other than ethyl alcohol; the presence of an aromatic aldehyde group is essential for the reaction as described in this paper; and finally of 104 substances tested by the technique, 15 samples of *Cannabis sativa* resin alone gave the reaction.

In this paper detection methods only are considered although it is believed that the test as carried out by the author using vanillin, salicylaldehyde, and *p*-dimethylaminobenzaldehyde could be developed into a quantitative colorimetric estimation.

The samples of *Cannabis sativa* resin examined in this investigation were obtained by the extraction with petroleum ether of ten separate samples of plant material of Indian origin and five samples of the crude drug seized by local custom and police officials. The color changes recorded in Table I were given by the crude resin, the resin after the passage through a Tswett chromatographic absorption column of activated alumina, and the resin after treatment with activated carbon.

Experimental

In Table I are recorded color changes using benzaldehyde, vanillin, *o*-nitrobenzaldehyde, *p*-dimethylaminobenzaldehyde, and salicylaldehyde.

The materials and reagents were tested in the following manner:

A volume of petroleum ether extract containing 2 mg. of the resin was either spotted onto a spotting porcelain plate with depressions, or added to a microtest tube and the solvent allowed to evaporate. A quantity of the aldehyde corresponding to 3 mg. contained in 0.5 cc. of the solvent was added to the resin and the material was dissolved by manipulation with a microspatula. Concentrated hydrochloric acid was then added drop by drop from a micropipet until maximum color intensity was developed.

The test was carried out also by smearing a minute portion of the resin on filter paper, adding a small drop each of benzyl alcohol and benzaldehyde, and developing the color with concentrated hydrochloric acid. The reaction under these conditions is very striking and is of value in the examination of stains and minute quantities of the resin.

Color Changes

In general under the conditions of testing described above benzaldehyde gave an intense violet color which faded rapidly. Vanillin gave a persistent green–blue-green coloration and in some cases violet tinges were noted. The blue-green color faded gradually, but in all cases the color was clearly perceptible after 20 minutes. *o*-Nitrobenzaldehyde,

Table I. Color Changes of *Cannabis sativa* Resin

Alcohol Solvent	Reagent	Color	Persistency	Remarks
Methyl alcohol	A	Violet	1 minute	Fades rapidly
	B	Green–blue green	Slow change	Perceptible green after 20 minutes
	C	Little change	.....	Yellow-green precipitate
	D	Violet red	Slow change	Cloudy violet after 20 minutes
	E	Deep green	Slow fade	Yellow green after 20 minutes
Ethyl alcohol	A	Violet	3 minutes	Fades in 5 minutes
	B	Deep blue green	Slow fading	Deep green after 20 minutes
	C	Brown yellow	Slow fading	Pale yellow after 20 minutes
	D	Violet red	Slow fading	Pink after 20 minutes
	E	Deep green	Slow fading	Green after 20 minutes
Isobutyl alcohol	A	Violet	5 minutes	Fades rapidly to slate
	B	Green–blue green	Slow fading	Blue green after 20 minutes
	C	Brown yellow	5 minutes	Slow fading to yellow
	D	Violet red	Slow fading	Pink after 10 minutes
	E	Deep green	Slow fading	Green after 20 minutes
Acetone	A	Cloudy violet	1 minute	Complete fading in 5 minutes
	B	Cloudy green	Slow fading	Light green after 20 minutes
	C	Cloudy brown	5 minutes	Yellow in 15 minutes
	D	Cloudy pink	Slow fading	Light pink after 20 minutes
	E	Cloudy green	Slow fading	Light green after 20 minutes
Benzyl alcohol	A	Violet	3 minutes	Fades to slate in 5 minutes
	B	Green–blue green	Slow fading	Green after 20 minutes
	C	Orange	Slow fading	Yellow after 20 minutes
	D	Violet red	Slow fading	Pink after 20 minutes
	E	Deep green	Slow fading	Pink after 20 minutes

A. Benzaldehyde. B. Vanillin. C. *o*-Nitrobenzaldehyde. D. *p*-Dimethylaminobenzaldehyde. E. Salicylaldehyde.

depending on the solvent used, gave light to brown-yellow colorations which persisted up to 20 minutes. With *p*-dimethylaminobenzaldehyde, beautiful violet-red colorations were developed which faded slowly, the color however being still perceptible after 20 minutes. With salicylaldehyde a beautiful emerald green color was developed immediately and persisted with slow fading for over 20 minutes. The color faded to a yellow brown in 2 hours. The color changes were not affected by the addition of acetaldehyde as recorded by Duquénois and Moustapha.

The reaction did not take place if aliphatic aldehydes replaced the aromatic aldehydes. When experiments were performed using acetaldehyde and formaldehyde in the manner described, in no case was color developed. Similarly ketones such as acetone and acetophenone fail to react. That the reaction requires the presence of a free aromatic aldehyde group is indicated with salicylaldoxime. When this oxime is used in place of the free aldehydes recorded above, no reaction takes place.

Effect of Solvent

The best solvents appeared to be benzyl alcohol and, in most cases, isobutyl alcohol. Ethyl alcohol gave fair results with all the aldehydes, but methyl alcohol and acetone were definitely inferior under the condition of test. This appeared to be due to either the insolubility of the reaction products in the solvents named or the cloudiness developed by the excess acid required to develop the maximum color in these solvents.

Effect of Acid

Neither sulfuric, nitric, nor acetic acid was able to develop color under the conditions of the test. With methyl alcohol and acetone as much as 1 cc. of acid was required to develop the color, whereas with isobutyl and benzyl alcohols 0.3 to 0.4 cc. was sufficient.

Specificity

These reactions, which are similar in type, appear to be specific for *Cannabis sativa* resin. Ghamrawy has tested 60 drugs, Munch 107 drugs, and Duquénois and Moustapha have also recorded the specificity of their technique. In this investigation several substances extractable from plant material with petroleum ether and other plant products which might appear in extracts were tested by the methods described above. The following substances failed to give the reactions recorded in Table I.

Alkaloids. Atropine, aconitine, berberine, brucine, caffeine, cinchonine, codeine, colchicum, cocaine, ecgonine, homatropine, hydrastine, morphine, narceine, nicotine, narcotine, papaverine, strychnine, theobromine.

Essential Oils. Rosemary, ti-tree, croton, verbena, vetiver, nutmeg, amygdalus, bay, citronella, lemongrass, eucalyptus, cajuput, lavender, clove, chenopodium, sandalwood, turpentine.

Fixed Oils. Candlenut, tung, coconut, linseed, mustard dilo, peanut, cottonseed, castor, sesame, cedarwood, olive, hydnocarpus.

Resins. Gurjun balsam, colophony, dammar, guiacum, Canada balsam, derris, kauri gum.

Terpenes and Sesquiterpenes. Pinene, cedrene, carvene, camphene, vetivene, cadinene.

Sugars. Dextrose, levulose, sucrose, mannose, lactose, galactose.

Unclassified. Meconic acid, crude opium, citronellol, citronellal, camphor, ethyl hydnocarpate, salicin, barbitone, nicotinic acid, phytosterol, carotene, ascorbic acid, abietic acid.

It is considered, therefore, that the reactions recorded in Table I take place with a constituent or constituents present in *Cannabis sativa* resin. The reactions have not been carried out as yet with material of known physiological activity, so that it is not possible to attribute the reaction to a physiologically active constituent or constituents.

Sensitivity

Although the color is fleeting, the most sensitive reagent is benzaldehyde in benzyl alcohol as solvent. A deep violet color is obtained immediately with amounts of the order of 0.03 mg. Definite reactions can be obtained with 0.1 mg. using vanillin, *p*-dimethylaminobenzaldehyde, and salicylaldehyde in benzyl alcohol. *o*-Nitrobenzaldehyde does not appear to be as sensitive as the other aldehydes. The slow fading in color of the vanillin, *p*-dimethylaminobenzaldehyde, and salicylaldehyde reaction products suggest the possibility of colorimetric determination, but this point has not as yet been investigated.

Conclusion

A definite chemical reaction takes place between one or more active constituents of Indian hemp and certain aromatic aldehydes dissolved in suitable solvents and in the presence of concentrated hydrochloric acid. The color changes are characteristic of the aldehydes and *Cannabis sativa* resin. Of the materials tested *Cannabis indica* resin alone reacted in the manner described.

Acknowledgment

The author is indebted to the Director of Agriculture for permission to publish these results.

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Determination of the Acetyl Group

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THE determination of acetyl content by means of trans-esterification in ethanol, followed by distillation and determination of the ethyl acetate so formed, was first described by Perkin (*3*), who employed sulfuric acid to catalyze the reaction. The original procedure has since been modified with a view to lessening the decomposition of residues and driving the reaction to completion, as well as assuring quantitative recovery of ethyl acetate.

Sudborough and Thomas (*5*) substituted aromatic sulfonic acids for sulfuric acid as catalyst, eliminating charring of residues together with attendant errors due to formation of sulfur dioxide during distillation. Freudenberg (*2*) employed an intermittent refluxing-distillation technique, adding fresh ethanol to the reaction flask after the first ester had been distilled off and repeating the refluxing-distillation sequence. Phillips (*4*) introduced alcohol vapors into the reaction flask from an external generator during the distillation. These methods require the accumulation of considerable volumes of distillate.

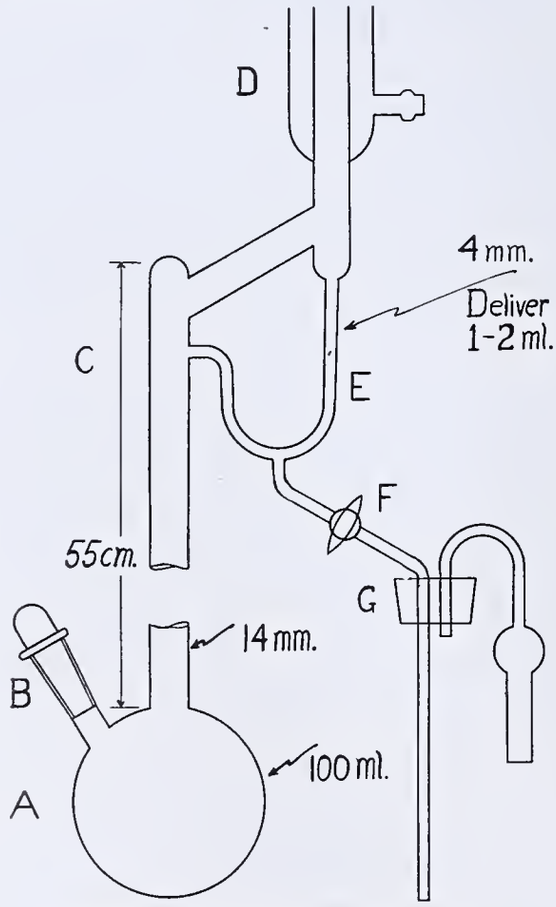

FIGURE 1. DIAGRAM OF APPARATUS

The method herein described accomplishes the removal of ethyl acetate in a minimum volume by distillation through an efficient fractionating column, arranged for operation under total reflux with intermittent small volume take-offs. A nearly constant volume is retained in the reaction chamber, thereby avoiding concentration of the acid in the solution, and enhancing the likelihood of recovery of the deacetylated residue if desired. Advantage is taken of the difference in boiling points of the ternary azeotrope ethyl acetate–ethanol-water (b. p. 70.2°) and alcohol (b. p. 78.3°), which is sufficiently large to permit ready quantitative separation. Concentrated aqueous hydrochloric acid is used as catalyst in place of the less readily available aromatic sulfonic acids or the objectionable sulfuric acid. It is equally effective under the specified conditions and no chloride resulting from the distillation of either hydrochloric acid itself or ethyl chloride may be detected in the hydrolyzed distillate.

TABLE I. RESULTS

Compound	Weight	Vol. of 0.1 *N* NaOH Consumed	Acetyl Found	Acetyl Calcd.
	Gram	*Ml.*	%	%
Diacetyl morphine HCl	0.8064	37.7	20.10	20.30
Calcium acetate.H_2O	0.3250	36.8	48.69	48.84
Aspirin	0.5025	27.8	23.79	23.88
	0.7247	40.2	23.85	
	0.7187	39.8	23.81	
	0.7243	40.3	23.93	
	0.7118	39.65	23.95	
Acetanilide	0.1900	14.0	31.68	31.85
	0.2038	14.95	31.54	
	0.2013	14.95	31.93	
	0.2015	15.0	32.01	
Acetphenetidine	0.2080	11.45	23.67	24.01
	0.1897	10.5	23.80	
	0.2018	11.2	23.87	
	0.2006	11.1	23.79	

The apparatus (Figure 1) used is simple and readily constructed, and the procedure involves a minimum number of operations. By suitable modification, the method can be adapted to semimicrooperation. The small final volume in the receiving flask, in contrast to that obtained by other procedures, permits use of an initially more dilute standard alkali solution for the saponification of the ethyl acetate, while retaining a sufficient final concentration to bring about the saponification. Objections to trans-esterification methods hitherto published, noted by Clark (*1*) on these grounds, are thus overcome.

Apparatus

A 100-ml. round-bottomed flask, *A*, fitted with a ⅋ 12/30 joint, *B*, serving as inlet is attached to a 14-mm. tube, *C*, 55 cm. in length, packed with 4-mm. single-turn glass helices for 50 cm. of its length. The condenser, *D*, is set off the top of this column, causing the condensate to return to the head of the column through the 4-mm. U-tube, *E*, of 1- to 2-ml. capacity. The take-off, *F*, consists of a tube and stopcock sealed into the lowest point of the U-tube and passing through a two-holed stopper, *G*, to which a 125-ml. Erlenmeyer flask is attached. The tube is almost long enough to reach the bottom of the latter flask. Through the second hole of the stopper is a vent protected by a soda-lime tube. The apparatus may be washed by flushing through the condenser, removing the wash-liquid through opening *B* by means of a tube attached to a suction flask.

Procedure

O-ACETYL. Introduce a suitable sample (equivalent to 10 to 30 ml. of 0.1 *N* alkali) into *A* through *B*, washing it in with a total of 50 ml. of pure absolute ethanol, previously freed from ester and aldehyde by refluxing and distillation over potassium hydroxide and aluminum powder. Add 2 ml. of concentrated aqueous hydrochloric acid and several Carborundum chips to serve as boiling points. Attach a 125-ml. flask containing an excess of 0.1 *N* sodium hydroxide solution to *G*, with the end of *F* extending below the surface of the solution. Immerse the receiving flask in an ice bath.

Distill the solution in *A* as rapidly as possible without flooding the column, keeping the stopcock in *F* closed. Drain *E* through the stopcock after each of eight 15-minute intervals. Remove the receiving flask, wash the outside of the tube with 5 ml. of ethanol, and allow 2 to 3 ml. of distillate to wash through the inside. Reflux the solution 0.5 hour. Cool and titrate the excess sodium hydroxide with 0.1 *N* acid, using phenolphthalein as indicator. Each milliliter of 0.1 *N* sodium hydroxide consumed is equivalent to 0.0043 gram of acetyl. A blank run on the alcohol should not consume more than 0.1 ml. of 0.1 *N* sodium hydroxide.

I-Acetyl. Proceed in the manner described above but use l. of hydrochloric acid as catalyst and space the take-offs at rvals of 30 minutes rather than 15.

The velocity of the trans-esterification reaction is, of course, endent on the structure of the compound concerned as l as the concentration of acid, and in general is greater for cetyl than for N-acetyl compounds. The time of re-ing and amount of catalyst indicated have been found able for the substances studied. These include acetphenetidine, which reacts with considerable difficulty; hence, it is presumed that the conditions specified would suffice for most compounds.

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Mineral Analysis of Biological Materials

Use of the Lundegårdh Spectrographic Method

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N 1929 and 1934 Lundegårdh (*1*, *2*) published the details of an accurate quantitative spectrographic method for the ermination of small quantities of metals by using an air-ylene flame into which was sprayed a solution of the sub-ice under investigation. Since that time, many improve-its in the apparatus (*4*, *10*) have simplified the procedure iout affecting the accuracy and reproducibility of the lts. This method of excitation for the production of emis-spectra has been shown to be very dependable, and more-it "is characterized by a remarkable absence of interac-s between the elements in the course of the analysis" (*9*). s is true of both anions and cations. The method has ved to be especially valuable for the analysis of biological stances of all kinds because, in comparison with chemical hods, it requires very small amounts of materials, and the erminations are more rapid and usually of a higher degree ccuracy.

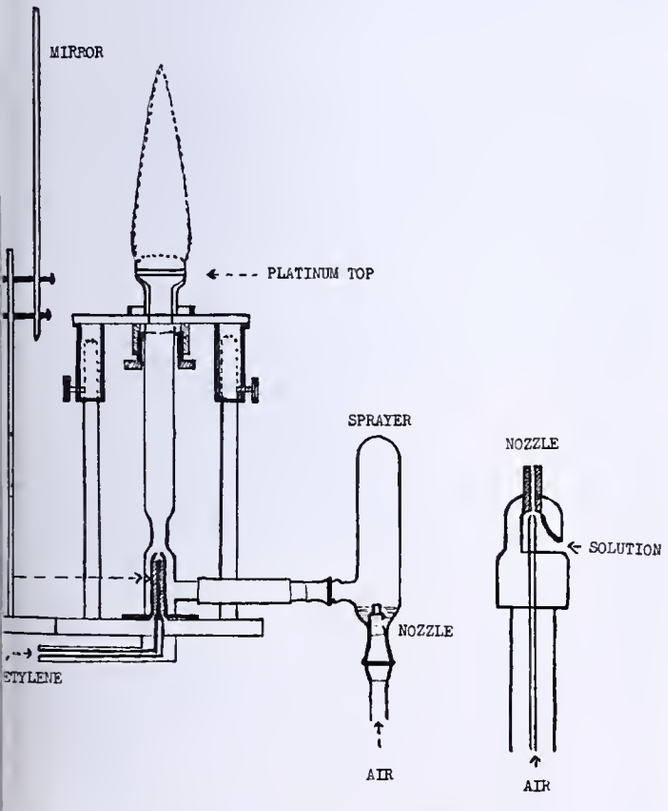

Figure 1. Diagram of Burner

In order to make a thorough study of the possible uses of the Lundegårdh technique, various types of problems have been investigated. At this time final reports on the special problems are not being made, but such results are included as are necessary to show the accuracy and adaptability of the method.

Apparatus

The apparatus consists of three distinct parts: a Lundegårdh air-acetylene burner and the sprayer through which the solution is introduced into the flame, a Hilger medium quartz spectrograph for photographing the spectra, and a Zeiss spectrum line photometer for measuring the intensity of the spectral lines.

The burner (*11*), sprayer, and nozzle (*6*) are shown diagrammatically in Figure 1. The inverted position of the sprayer permits the use of as little as 2 ml. of the solution.

The arrangement of the burner and the spectrograph is shown in Figure 2. In the background are the two manometers that indicate accurately the air and acetylene pressures, which can be controlled by slight adjustments of the reducing valves on the cylinders. The rack at the left is used for draining the sprayers, and the switch box, shown on the table, controls the solenoid which operates the shutter in front of the slit.

The burner is set up about 45 mm. in front of the slit and exactly in the line of the optical axis of the spectrograph. The intensity of the light entering the slit is greatly increased by the reflection of the flame from a mirror, which is placed so that the reflected image is also in the line of the optical axis of the spectrograph. Lundegårdh found that the most complete emission of most elements takes place in the part of the flame 15 mm. above the top of the inner blue cone (*4*). For this reason the height of the burner is adjusted so that this portion of the flame is directly in front of the slit.

Experimental Procedure

The conditions which produce the maximum intensity of the lines without too much background are obtained by exposures of 2 minutes with an air pressure of 26,366 kg. per square meter (37.5 pounds per square inch) and an acetylene pressure of about 26 cm. of water. Compressed air and acetylene are used from the cylinders as purchased. The slit width is 0.05 mm. and the length of the slit is only 1 mm., so that forty-two exposures can be made on each plate.

Eastman plates No. 33 are used. They are developed for 6 minutes in a tank with Eastman high-contrast developer, formula D-19, and fixed for 12 minutes with Eastman acid fixer, formula F-5.

The intensities of the spectral lines are determined by examination of the plate in the microphotometer. A device for reading "near the line" similar to that used on the Lundegårdh photometer (*7*) has been attached to the Zeiss instrument. The ratio of the reading of the line to the reading of the background is called the intensity ratio (*3*, *5*) and is used for the construction of the intensity ratio–concentration curve. The values for the concentrations are those of four different standard solutions whose

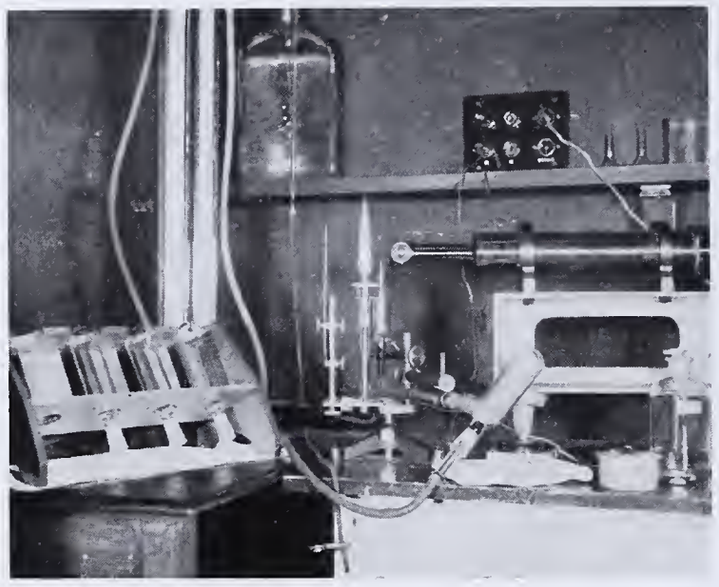

FIGURE 2. SET-UP OF BURNER

spectra are photographed on every plate. Two exposures are taken of every solution and the average of the two intensity ratios is used. At three different intervals on the plate, the spectrum of one of the standards is repeated; this makes it possible to construct a correction curve if the values show any variations that might be due to emission conditions or to the plate emulsion.

In Table I the wave lengths and the maximum and minimum concentrations which are suitable for the construction of the concentration–intensity ratio curves, expressed both in molarity and in grams per liter of solution, are given for each of the metals used.

Some typical intensity ratio–concentration curves are shown in Figure 3.

Accuracy

In order to investigate the accuracy of this method, solutions of known concentrations of a number of the metallic ions were prepared and the ions then determined by the Lundegårdh method, using a single plate, with the results presented in Table II.

Table I. WAVE LENGTHS AND CONCENTRATIONS OF METALS

Metal	Molarity		
	Å.		*Gram/l.*
Calcium	4226.7	0.0000125–0.0001	0.00050–0.0040
Cobalt	3526.8	0.00025–0.002	0.0147–0.1179
Copper	3247.5	0.000025–0.0002	0.00159–0.0127
Iron	3720.1	0.00025–0.002	0.01395–0.1116
Magnesium	2852.1	0.00025–0.002	0.0061–0.0486
Manganese	4030.8	0.0000125–0.0001	0.00069–0.0055
Nickel	3524.5	0.00025–0.002	0.0147–0.1174
Potassium	4044.2	0.00025–0.002	0.0098–0.078
Silver	3383.9	0.000025–0.0002	0.0027–0.0216
Sodium	3302.3	0.0005–0.004	0.0115–0.0920

TABLE II. COMPARISON OF WEIGHTS OF ELEMENTS PRESENT WITH WEIGHTS FOUND BY LUNDEGÅRDH METHOD

	Calcium	Copper	Iron	Magnesium	Manganese	Potassium	Sodium
	Gram/l.	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*
Present	0.00301	0.0095	0.0837	0.0365	0.00412	0.0587	0.0690
Found	0.00299	0.0092	0.0822	0.0356	0.00412	0.0583	0.0690
	0.00302	0.0092	0.0860	0.0352	0.00408	0.0535	0.0682
	0.00296	0.0090	0.0850	0.0341	0.00413	0.0561	0.0674
Present	0.00140	0.0045	0.0391	0.0170	0.00192	0.0274	0.0322
Found	0.00142	0.0042	0.0404	0.0178	0.00192	0.0264	0.0345
	0.00140	0.0047	0.0366	0.0158	0.00192	0.0284	0.0295
	0.00135	0.0044	0.0390	0.0158	0.00199	0.0270	0.0333
Present	0.00060	0.0019	0.0167	0.0073	0.00082	0.0117	0.0138
Found	0.00057	0.0017	0.9166	0.0065	0.00078	0.0111	0.0123
	0.00062	0.0022	0.0181	0.0081	0.00087	0.0111	0.0134
	0.00065	0.0017	0.0166	0.0083	0.00094	0.0091	0.0130

Preparation of Materials

Since this method demands that the metallic elements in solution, the preparation of material for this analysis i matter of great importance. In the first place accurat calibrated volumetric apparatus and chemicals of the high purity are essential and have been used throughout this wo Spectrographic tests were made on concentrated solutions u in, the preparation of all standard solutions and reagents ensure the absence of impurities.

In order to avoid a difficulty frequently encountered in c relating the results of several investigators—namely, the certainty of the moisture content of the initial material study of possible methods of moisture determination has b made. The following procedures have been adopted:

Solid materials are pulverized, weighed, and dried to const weight at 70° C. Those substances which become darkenec charred at this temperature are spread in thin layers in large weighing bottles and allowed to stand in desiccators over c centrated sulfuric acid until the weight is constant. This usu requires about 72 hours. The samples of liquids such as ora juice or blood are weighed, and also when possible the volu are measured. Thus the final results can always be defini expressed in relation to the original material.

Various methods for the actual treatment of the mate have been tried and digestion with nitric acid and Perhyc has been found to be very satisfactory. Complete oxidat of even large quantities of organic material can be acc plished in relatively short periods and the residues are rea soluble in water or in very dilute nitric acid.

The method adopted for all purposes has been to weigh material directly into 70-ml. Pyrex test tubes calibrated at 20 Nitric acid is added and the tubes are then heated in boiling w until any moisture present, as in orange juice, is nearly evapor off. The tubes are then heated in a concentrated sulfuric bath at a temperature between 120° and 140° C. until the solu is clear. Perhydrol is next added and the tube is carefully he over a microburner. If necessary, small additional portion nitric acid and Perhydrol are added to decolorize the solu completely and the solution is finally evaporated nearly to ness. It can then be diluted to the 20-ml. mark or transfe to any small volumetric flask and diluted to volume. It ma used both for the spectrographic determination of the t metals and for the various dilutions needed for the more abun elements present.

Complete oxidation of organic matter is important bec it has been found that a residue may contain practically a the calcium and cannot safely be discarded. Lundeg (*8*) has shown that the presence of nitric acid of this con tration in these solutions does not affect the spectrogra determinations of the metals. "Sulfuric acid and nitric at a concentration of 1 mole had no perceivable effect u any cation."

Several other methods for the preparation of the mat were studied and abandoned. Dry-ashing at 400° was companied by a loss of about 30 per cent of the total potass and by as much as 10 per cent of the cium. Extraction by refluxing molar hydrochloric acid and centrifu or filtering off the organic residue excellent results for the more plen elements, but could not be used fo trace elements. A similar extrac with nitric acid showed repeatedly the calcium was very largely adsorbe the residue. The use of any Kjel method was vitiated by the l quantity of sodium extracted from Pyrex glass (*13*).

Since the preparation of various m rials for analysis may involve slight l of some of the metals, the possibl

ery of such substances, added in known amounts at the inning of the experiment, was studied. Table III shows h results for some metallic ions added to 50-ml. portions range juice.

For a dry material the recovery was tested by adding 0023 gram of calcium ion to a 2.4032-gram sample of ter beans before wet-ashing. The calcium recovered was 0020 gram.

BLE III. RECOVERY OF METALS ADDED TO ORANGE JUICE

	Metal Average	Added Metal	Total Metal Found	Added Metal Recovered
	Gram/l.	*Gram/l.*	*Gram/l.*	*Gram/l.*
ium	0.156	0.274	0.4365	0.281
		0.274	0.4320	0.276
		0.274	0.4290	0.273
ssium	2.61	4.69	7.49	4.88
		4.69	7.23	4.62
		4.69	7.49	4.88
nesium	0.110	0.199	0.290	0.180
		0.199	0.290	0.180
		0.199	0.299	0.189
um	0.0152	0.0230	0.0383	0.0231
		0.0230	0.0375	0.0223
		0.0230	0.0356	0.0204

Applications

The plant materials investigated were mainly oranges, ter beans, and pectinates. Much of the preliminary work s done on oranges furnished by the Agricultural Experi- nt Station at Orlando, Fla. Triplicate analyses of one iety of Temple oranges are given in Table IV. It is of erest to compare these results with those of Roberts and ddum (*12*).

TABLE IV. ANALYSIS OF TEMPLE ORANGES

(Grams of metal per liter of juice and pulp)

Calcium	Copper	Iron	Magnesium	Manganese	Potassium	Sodium
Gram/l.	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*
0.158	0.000465	0.00198	0.112	0.000372	2.00	0.0201
0.153	0.000466	0.00187	0.108	0.000380	2.06	0.0194
0.156	0.000451	0.00209	0.104	0.000377	2.25	0.0218

This method was also found useful for the analysis of arium and natural sea waters and has been successfully lied to animal tissues, lenses of rat eyes, bones, and both ole blood and serum. Rat blood samples ranging from to 1.7 grams were quantitatively transferred from the ghing bottles to the digestion tubes by treatment with nitric acid and distilled water and the usual wet-ashing cedure was followed. One gram of blood required about l. of acid and about 20 drops of Perhydrol for complete dation. Table V gives the results of triplicate determina- ns made on an experimental rat which probably had de- oped anemia.

Summary

The Lundegårdh air-acetylene burner used for the excita- n of the emission spectra of metallic substances in solution lescribed, together with the Lundegårdh method of calcu- lation of the concentration of these ions in the solutions used. A series of results for calcium, copper, iron, magnesium, manganese, potassium, and sodium shows a very satisfactory recovery of these elements when solutions containing known concentrations are analyzed.

Methods of drying the biological materials are given, followed by a discussion of methods of preparation of these substances for analysis. Complete oxidation with nitric acid and Perhydrol at 120° to 140° C. is recommended. Recovery of metals added to these materials before the above treatment is excellent.

The wide applicability of the method is evident from its successful use in the analysis of such substances as oranges, beans, pectinates, aquarium water, rat lenses, bones, and blood.

TABLE V. ANALYSIS OF RAT BLOOD

(Per cent by weight in whole blood)

at o.	Weight of Sample	Calcium	Sodium	Iron	Potassium
	Gram	%	%	%	%
	0.3444	0.00505	0.200	0.0358	0.2065
	0.3440	0.00515	0.198	0.0359	0.2045
	0.3979	0.00504	0.189	0.0382	0.1855

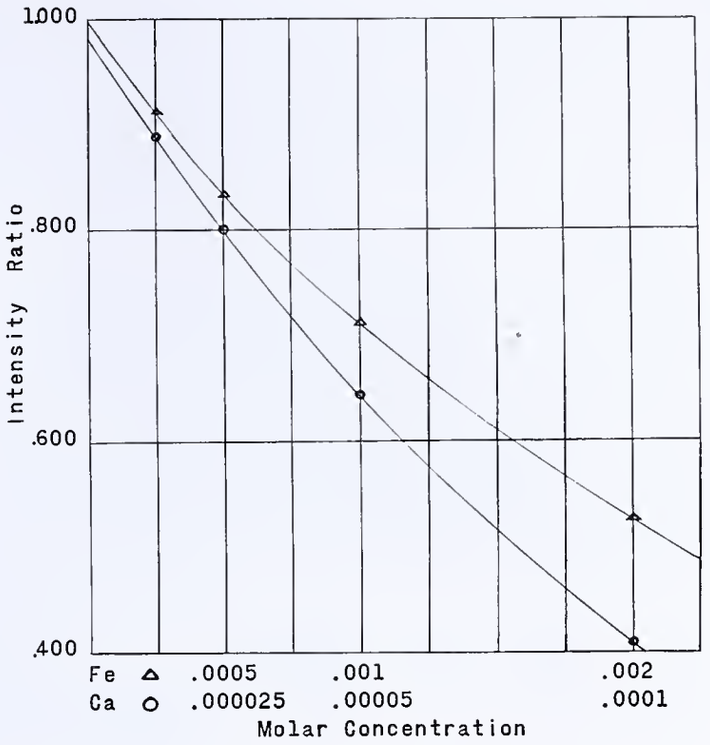

FIGURE 3. CONCENTRATION–INTENSITY RATIO CURVES FOR CALCIUM AND IRON

Acknowledgment

The authors wish to acknowledge their indebtedness to Miss Ruth Abbott for her share in the experimental work.

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Determination of Metals in Some Pectinates

Comparison of Photometric and Spectrographic Methods

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DURING certain biological investigations, it became necessary to determine the metal content of cobalt, nickel, and bismuth pectinates and of preparations containing these substances. (The pectinates were obtained from the Sardik Company and the nickel pectinate gel from Eli Lilly and Company.) Since a Zeiss-Pulfrich photometer for colorimetric measurements and the complete spectrographic equipment for analysis by the Lundegårdh (*2*, *3*, *4*) method were available, these two methods were studied in order to compare their agreement when used for small quantities of the materials.

TABLE I. AVERAGE PER CENT MOISTURE IN PECTINATES

	Average Moisture, %
Nickel pectinate I	7.64
Nickel pectinate II	7.91
Nickel pectinate III	4.53
Nickel pectinate IV	5.52
Nickel pectinate gel	70.28
Cobalt pectinate	9.43
Bismuth pectinate	7.55

Experimental Methods and Results

As it was found possible to dry the materials to constant weight at 70° C. without any change in appearance, this temperature was used for the determination of moisture.

In general, when the dry material was prepared for analysis by ashing at 500° C. before treatment with nitric acid, there was a slight loss of the metals. For this reason and also because the percentage of total ash was not important, all solutions were prepared by wet-ashing with concentrated nitric acid at 125° to 145° C. until all organic material was destroyed (*1*). Most of the acid was then expelled, and after necessary dilutions were made the solutions were ready for analysis.

The Lundegårdh spectrographic method as used in this study has been described in detail by Griggs, Johnstin, and Elledge (*1*). The spectrum lines used for nickel and cobalt were 3524.5 and 3526.8 Å., respectively. The concentration curves for both the nickel and cobalt were constructed from standard solutions containing from 0.002 to 0.00025 mole per liter. The results of these analyses are given in Tables II, III, and IV. As Lundegårdh found, this method of excitation does not produce spectrum lines suitable for the determination of bismuth, so that no spectrographic results could be obtained.

NICKEL. The photometric method used by Murray and Ashley (*6*) for the determination of nickel in steel was chosen for the analysis of nickel pectinates. The concentration curve was prepared by the use of solutions containing 0.2560 to 0.0512 mg. of nickel. To each solution citric acid, saturated bromine water, ammonia, and dimethyl glyoxime were added before dilution to the final volume of 100 ml. The nickelic complex solution has a pale wine color. It was found necessary to take the photometer readings after 5 minutes had elapsed and to complete them within the next 5 minutes in order to avoid change in transmission.

Nickel pectinates I and II were found to contain some silica, which evidently was precipitated when ammonia was added and adsorbed the colored nickel complex. It was therefore necessary to remove the silica by treatment wit hydrofluoric and sulfuric acids before making the photometr determinations.

COBALT. The photometric method developed by MacPhers and Stewart (*5*) for the determination of cobalt was adopte The concentration curve was prepared by the use of solutio containing 0.02585 to 0.002585 mg. of cobalt. The cob was oxidized by nitric and hydrochloric acids and a compl yellow cobaltic compound was formed by the addition of nitros R salt and sodium acetate with careful control of temperature a pH of the solution, the final volume of which was exactly 25 m Since the color was not permanent, it was necessary to make photometer readings at a definite interval (such as 1 hour) aft the addition of the dye. It was also found that the concentr tion-transmission curve passed through the origin only when blank solution containing all reagents was used in the co parison cup instead of water.

BISMUTH. For the analysis of bismuth pectinate the colo metric method of Rasmussen, Jackerott, and Schou (*7*) w adapted for use with the photometer. It had been found th dilute solutions of bismuth iodide were yellow in color and th their light absorption was proportional to the concentration the bismuth. Filter S47 and 20-mm. cups were used in t photometer and solutions containing 0.1820 to 0.0455 mg. bismuth were prepared for the construction of the concentrati curve. In the case of these solutions as well as solutions of t pectinate, prepared as described above, it was necessary to move nitric acid by evaporation in order to prevent oxidation the iodide. One drop of sulfuric acid and 10 ml. of a 10 per ce

TABLE II. NICKEL IN NICKEL PECTINATES

Nickel Pectinate	Weight of Sample	Nickel Found: Photometric method	Nickel Found: Spectrographic method
	Gram	%	%
I	0.2272	0.22	0.27
	0.1994	0.24	0.26
	0.2161	0.21	0.27
	0.2475	0.19	0.24
		Av. 0.22	0.26
II	0.3554	0.27	0.25
	0.2653	0.26	0.27
	0.1884	0.26	0.25
	0.3093	0.27	0.26
	0.3256	0.28	0.25
		Av. 0.27	0.26
III	0.2299	0.46	0.48
	0.2131	0.43	0.41
	0.2631	0.44	0.41
	0.1693	0.45	0.43
	0.2159	0.45	0.44
		Av. 0.45	0.43
IV	0.2221	0.68	0.68
	0.2840	0.68	0.68
	0.1603	0.67	0.62
	0.1433	0.66	0.65
	0.1822	0.67	0.67
		Av. 0.67	0.66
Gel	20.4860	0.0048	0.0041
	20.0854	0.0046	0.0043
	18.8688	0.0046	0.0044
	19.9490	0.0048	0.0039
	19.4773	0.0047	0.0045
		Av. 0.0047	0.0042

TABLE III. COBALT IN COBALT PECTINATE

Weight of Sample	Cobalt Found: Photometric method	Cobalt Found: Spectrographic method
Gram	%	%
0.2997	0.42	0.36
0.2791	0.41	0.34
0.2111	0.36	Not determined
0.2891	0.37	Not determined
	Av. 0.39	0.35

solution of potassium iodide were then added before diluting to a volume of 25 ml. The concentration was plotted against per cent transmission on a semilogarithmic scale; the graph was a straight line passing through the ordinate at 100 per cent, showing that Beer's law held for these dilute solutions, and that the compound was suitable for use in the photometer.

Table IV. Bismuth in Bismuth Pectinate

Weight of Sample	Bismuth Found by Photometric Method
Gram	%
0.2155	0.57
0.2660	0 53
0.1801	0.53
0.2540	0 55
0.2834	0.55
	Av. 0.55

Conclusions

The photometric methods presented in this paper show excellent agreement and therefore high precision for the determination of very small quantities of nickel, cobalt, and bismuth in organic materials. The spectrographic method used for nickel and cobalt has almost as good agreement and precision but requires somewhat larger quantities of the materials. The latter is more rapid, however, if many samples are to be analyzed. The close agreement of the analyses by the two methods indicates that the results are very accurate.

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Use of Thorium Nitrate to Distinguish between Pectin and Certain Gums

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IDENTIFICATION of the individuals commonly grouped as water-soluble gums is at times difficult, as many of the substances react alike to the same reagent. Pectin is one of the most difficult of this group to identify, because it is usually precipitated by the reagents which also precipitate the other gums. Differentiating between pectin and other gums (*2*, *3*) requires more time and labor than a simple precipitation test. In attempting to carry out work on pectin similar to that done by Bonner (*1*) on pectate it was found that precipitation with thorium nitrate exhibits a novel property of pectin not shown by the other gums tested.

If the substance under examination is a powder containing no added materials, such as sugar or acid, it is made up to a 1 per cent aqueous sol. Any admixture should first be extracted with 50 per cent alcohol, or can be eliminated by dissolving the substance and then precipitating with an equal volume of alcohol. If the sample is a liquid preparation, the gum should be precipitated as above, so that a pure sol of known strength can be made.

Irish moss, agar, gum arabic, and karaya gum give no precipitate with 50 per cent alcohol; gum tragacanth gives only a slight precipitate; but pectin, quince seed, and locust-bean gums give good precipitates.

Of the gums listed in Table I, only Irish moss, quince seed, and pectin give precipitates with thorium nitrate. The precipitate from Irish moss is stringy and opaque and easily differentiated, but those from quince seed and pectin are alike, being of a firm, gelatinous nature. The pectin precipitate is easily dispersed or in some cases actually dissolves on adding an excess of the thorium nitrate solution or dilute acetic acid; the quince-seed precipitate with thorium nitrate is unaffected by this treatment. If the dilute acetic acid is first added to the gum sol, the quince seed will yield a firm gel and the pectin shows only a thickening or very slight gel.

A confirmation of the thorium nitrate test for pectin may be made with neutral lead acetate. In unacidified sols pectin and quince seed yield gels, while if acetic acid is added to the sol before the lead acetate, pectin forms a firm, clear, brittle gel but quince-seed gum gives only a very weak gel or a viscous sol. This is just the reverse of the thorium nitrate test.

Table I. Reactions of Gums and Related Substances with Thorium Nitrate and Neutral Lead Acetate

Material Used	Thorium Nitrate		Neutral Lead Acetate	
	10% solution	10% solution and 5 *N* acetic acid	10% solution	10% solution and 5 *N* acetic acid
Gum arabic	[a]	[a]	[a]	[a]
Locust-bean gum	[a]	[a]	Slight thickening	[a]
Gum tragacanth	[a]	[a]	[a]	[a]
Irish moss	Stringy, white precipitate	White granular precipitate	Cloudy	Cloudy
Karaya gum	[a]	[a]	[a]	[a]
Quince-seed gum	Firm opaque gel	Firm gel	Fairly firm gel	Very weak gel, a thickening
Pectin	Firm, transparent gel	Very weak gel, a thickening	Firm, transparent gel	Firm, brittle, clear gel
Agar (0.5 instead of 1%)	Slight haze	[a]	[a]	[a]
Methyl cellulose	[a]	[a]	[a]	[a]
Starch	[a]	[a]	[a]	[a]

[a] No apparent reaction.

To 10 ml. of the aqueous solution of the gum (1 in 100) add 1 ml. of 10 per cent thorium nitrate solution, stir, and allow to stand 2 minutes. If a gel results, the gum is either pectin or quince-seed gum. If no gel results it is not pectin.

To differentiate between the two gums: To 10 ml. of the sol add 1 ml. of 5 *N* acetic acid, then 1 ml. of 10 per cent thorium nitrate solution, stir, and allow to stand 2 minutes. If no firm gel results the gum is pectin; if a gel forms it is quince-seed gum.

To check the reaction, a 10 per cent solution of neutral lead acetate is used and the same procedure carried out as for thorium nitrate.

Table I shows the gums tested and the results obtained.

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Determination of Oxygen in Tank Hydrogen

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IN RESEARCH employing tank hydrogen it is often desirable to determine the presence of traces of oxygen. A method is here described for measuring these traces with an error of less than 10 per cent for oxygen concentrations of 0.05 to 0.20 per cent by volume. While the principle involved is far from new (*1*), the apparatus shown in the figure is considerably simpler and more accurate than those previously described. In principle, the traces of oxygen are burned with hydrogen on a glowing platinum wire. The water which forms condenses, thereby effecting a disappearance of three volumes, two of hydrogen and one of oxygen, for each volume of oxygen originally present.

APPARATUS. The whole apparatus is mounted on a wooden base 37.5 × 25 cm. (15 × 10 inches) with a backboard of the same dimensions. Bulbs *C*, *D*, *F*, and *G* are all of 100-ml. capacity. The slanting manometer, *E*, is of 2-mm. capillary tubing fastened to a 20-cm. length of meter stick, which is inclined to give a drop of 2 cm. over its 20-cm. length. A capillary stopcock may be conveniently inserted at *E*, although this is not essential. The water jacket surrounding *C* is made of 50-mm. tubing and a cork. A rubber stopper, rather than a glass-tungsten seal, is provided for the ignition vessel, *D*, so that the platinum wire can be easily replaced if burned out. Two stout tungsten lead-in wires are insulated from each other by a 5-mm. glass tubing, not shown, which is embedded in the rubber stopper and extends up over one wire to the platinum coil. The platinum spiral is made from No. 28 wire, 20 cm. long.

A scratch is made on *BD* about 3 cm. from *B*. *A* and *B* are small, two-way capillary stopcocks. Section *AI* should be bent out from the board. Leveling bulb *F* is set in a 5 × 5 cm. (2 × 2 inches) wooden block, hollowed out and filled with plaster of Paris, so that *F* may be returned to exactly the same position each time. The block is fastened to a rod which may be clamped into position. Bulb *G* rests in an ordinary ring. *CE* is filled with mercury and *D* with water. Bulb *C* and manometer *E* must be kept clean and dry at all times.

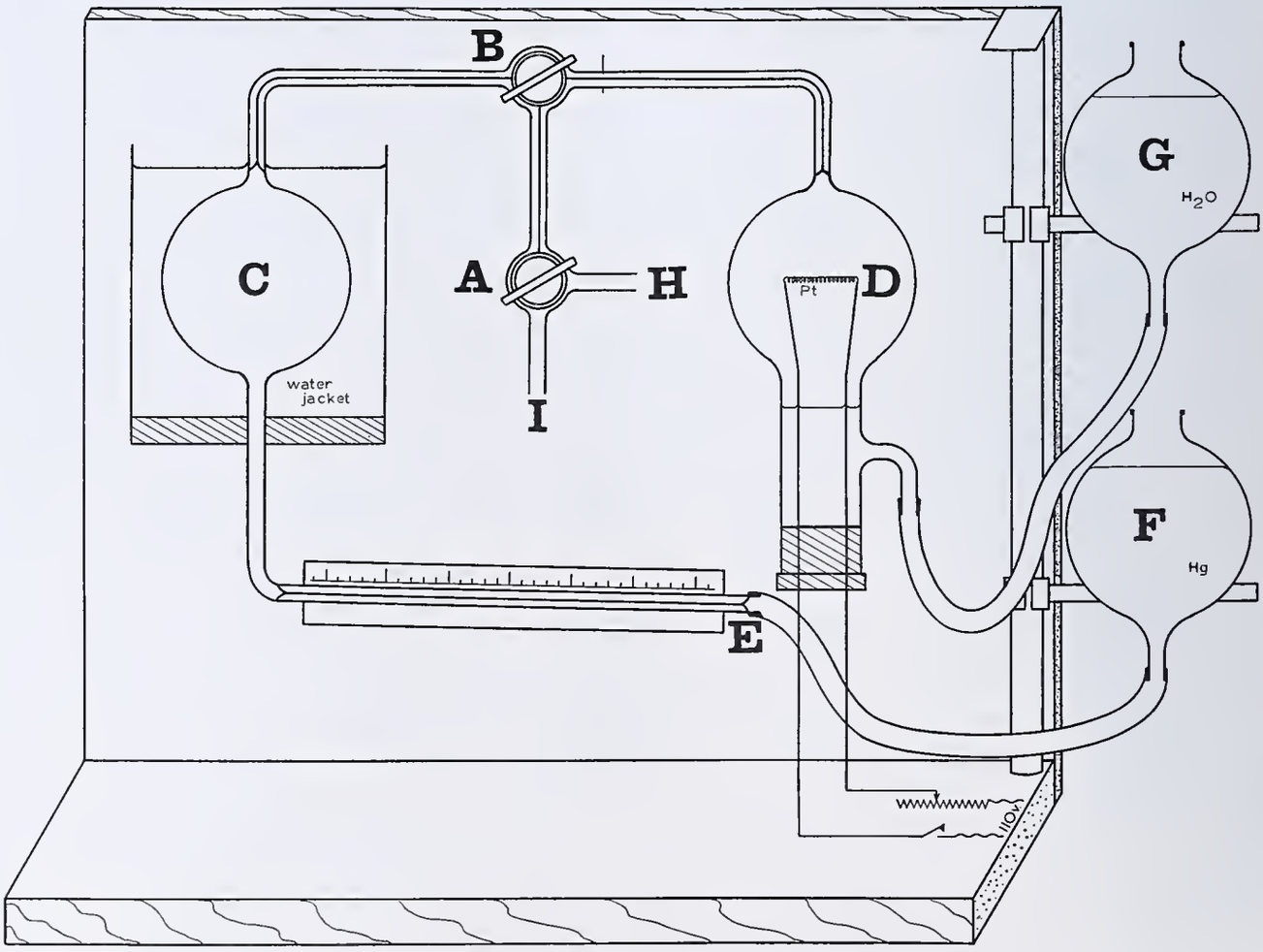
PRELIMINARY OPERATIONS. The residual air in the apparatus must first be replaced by tank hydrogen. Once this has been attained the steps in this section may be omitted.

Open passage *DBI* and raise *G* until the water nearly reaches *B*. Open passage *CBI* and raise *F* until the mercury gushes into *BI*. Excess mercury may be caught in a beaker held at *I*. Attach the hydrogen sample at *H*, open *HAI*, and flush out with the hydrogen. Then turn the cocks to open passages *HABC*, filling *C* with hydrogen. Flush out the residual air in *BD* by opening *CDB*, running in hydrogen by raising *F* or lowering *G*, and finally opening *DBAI* and *CBAI*. Both water and mercury will now be a few centimeters from *B*, and the tiny volume of gas between them will be nearly pure hydrogen. Therefore all subsequent measurements may omit these preliminary operations.

MAKING AN ANALYSIS. Open *CBAI*, and raise *F* to empty the gas out of *CB* until mercury again spills out at *I*. Open *HAI* to flush stopcock; then open *HABC* and run in the sample to be analyzed, stopping when the mercury reaches a point near *E* on the manometer. At this point, switch to *HAI*, and finally close

TABLE I. CONSECUTIVE READINGS ON MIXTURES

Composition of Gas by Volume Hydrogen	Oxygen	Movement in Manometer	Oxygen Found
%	%	*Mm.*	%
99.949	0.051	27	0.054
99.900	0.100	48	0.096
99.864	0.136	70	0.140
99.937	0.063	30	0.060
99.906	0.094	50	0.100



top cocks *A* and *B*. Record the position of the mercury on he manometer scale. Open passage *CBD*, and run the sample ver into *D*, stopping when the mercury is a few centimeters rom *B*. The water level will be well below the platinum wire. lose *B* and switch on the electricity, keeping the platinum spiral eated to a dull red, not white, heat. With hydrogen containing bout 0.05 per cent oxygen 10 seconds are sufficient; for 0.20 er cent oxygen heat for 1 minute, but never any longer. Allow he gas to cool for 2 minutes, return it to *C*, stopping the water at he scratch 3 cm. from *B*, and record the new manometer reading. he calibration of the manometer can be calculated from its slant, but it is simpler to make a direct measurement with empirical mixtures of oxygen and hydrogen.

Table I shows that a movement of the mercury along 1 mm. of the manometer corresponds to 0.002 per cent of oxygen by volume in the sample.

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Tungsten-Nickel and Tungsten-Silver Electrode Systems in Neutralizations

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THE bimetallic electrode systems, tungsten-nickel and tungsten-silver, were examined to ascertain their value or precise work in neutralizations involving dilute solutions. Kahlenberg and co-workers (*1*, *4*, *5*) have especially recommended tungsten as an indicator electrode for neutralizations, nd among the bimetallic electrode systems suggested by hese investigators for such reactions are tungsten-nickel nd tungsten-silver. After a precise study of the tungsten-ickel system in neutralizations Furman and Low (*3*) reported this system of value in titrations of strong acid with trong base, and *vice versa*, for solutions 0.1 *N* and above. According to their observations nickel undergoes an abrupt hange in potential at a pH about that at which the transition f methyl orange occurs, but is relatively insensitive in the egion at which phenolphthalein changes.

It is the purpose of the present paper to show that these haracteristics of the nickel electrode make the tungsten-ickel pair unsuitable for precise work in the titration of solu-ions as dilute as 0.01 *N*, and to describe the results of a letailed study of the tungsten-silver system in neutraliza-ions involving solutions the approximate concentrations of which are between *N* and 0.001 *N*.

Materials and Procedure

Solutions of about the normality desired were prepared from reagents of analytical grade, the alkali solutions being nearly free of carbonate and the usual precautions being taken to protect hem from atmospheric carbon dioxide.

Electrodes were of the following B. & S. wire gages: tungsten, 19; nickel, 8 and 24; silver, 24. After each titration the elec-rodes were cleaned with sandpaper, then washed with 6 *N* nitric acid and several changes of distilled water. Occasional omission of the nitric acid washing seemed to have no bearing upon the results.

A definite volume, between 15 and 40 ml., of the solution to be titrated was placed in a closed container and the neutraliza-tion was carried out at room temperature in an atmosphere of nitrogen free of carbon dioxide. While the solution was being stirred by a motor-driven stirrer, the course of the titration was followed by means of a Leeds & Northrup students' type poten-tiometer and accessories. Readings were taken after the e. m. f. appeared virtually constant. In the neighborhood of the end point these were recorded after each drop of the solution was added. The maximum of the Δ e. m. f./Δ ml. was taken as the electrometric end point and compared with end points obtained either simultaneously or independently with phenolphthalein, methyl orange, or methyl red indicators. The color change for methyl red was made more distinctive by the presence of methylene blue. Each of the end-point ratios (ml. of acid per ml. of base) tabulated in the following section represents the mean of values derived by three or more titrations. The average deviation from this mean is also given for the electrometric end-point ratios.

In many of the titrations employing the tungsten-silver system, the potential of each electrode was checked repeatedly against a reference electrode, a 0.1 *N* calomel cell—i. e., after each addition of reagent in the course of a titration, e. m. f. readings were taken for the systems tungsten-silver, tungsten-calomel, and silver-calomel.

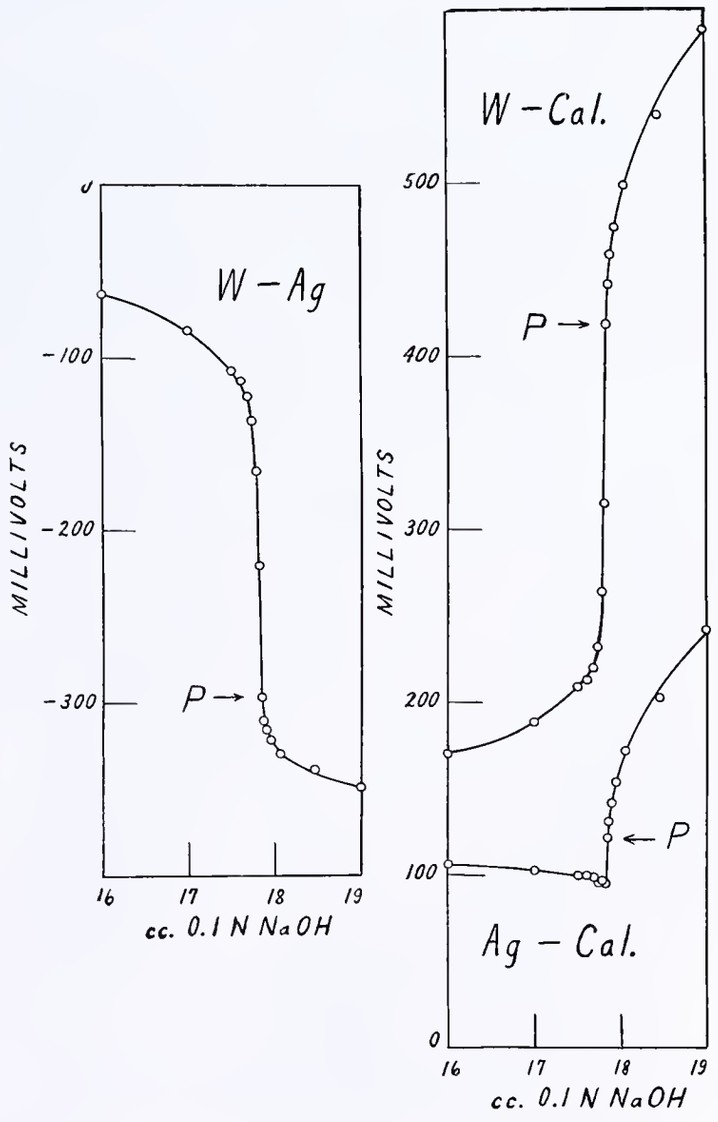

FIGURE 1. TITRATION OF 0.1 *N* HYDROCHLORIC ACID

Using tungsten-silver, tungsten-calomel, and silver-calomel system

TABLE I. TUNGSTEN-NICKEL SYSTEM

(Solution added: NaOH, normality approximately that of solution being titrated)

Solution Titrated (Approximate Normality)	End-Point Ratios: Methyl orange	Electrometric	Phenolphthalein	Electrometric
		Ml. acid per ml. base		
0.1 *N* HCl	1.136	1.138 ± 0.002	1.131	1.130 ± 0.001
0.1 *N* H_2SO_4	1.128	1.129 ± 0.004	1.118	1.120 ± 0.002
0.01 *N* HCl	...	1.062 ± 0.003	1.015	1.037 ± 0.01
0.01 *N* H_2SO_4	...	1.069 ± 0.003	1.043	1.045 ± 0.01

TABLE II. TUNGSTEN-SILVER SYSTEM

Solution Titrated (Approximate Normality)	Solution Added (Approximate Normality)	End-Point Ratios: Methyl red	Phenolphthalein	Electrometric
			Ml. acid per ml. base	
N HCl	*N* NaOH	...	1.162	1.162 ± 0.001
N H_2SO_4	....		0.7745	0.7741 ± 0.0006
0.1 *N* HCl	0.1 *N* NaOH	...	0.8380	0.8388 ± 0.0006
0.1 *N* H_2SO_4	....		0.8294	0.8297 ± 0.0006
0.01 *N* HCl	0.01 *N* NaOH	...	1.037	1.041 ± 0.001
0.01 *N* H_2SO_4	....		1.060	1.064 ± 0.002
0.001 *N* HCl	0.001 *N* NaOH	...	...	1.007 ± 0.002
0.001 *N* H_2SO_4	....	...	...	1.088 ± 0.002
N NaOH	*N* HCl	...	1.156	1.156 ± 0.001
....	*N* H_2SO_4	...	0.7726	0.7726 ± 0.0003
0.01 *N* NaOH	0.01 *N* HCl	...	1.037	1.043 ± 0.001
....	0.01 *N* H_2SO_4	...	1.060	1.063 ± 0.001
0.1 *N* HCl	0.1 *N* NH_4OH	0.9431	....	0.9429 ± 0.0003
0.1 *N* H_2SO_4	....	0.6067	....	0.6070 ± 0.0003
0.01 *N* HCl	0.01 *N* NH_4OH	1.131	....	1.132 ± 0.001
0.01 *N* H_2SO_4	....	1.685	....	1.685 ± 0.004
N $HC_2H_3O_2$	*N* NaOH	...	0.7863	0.7863 ± 0.0006
0.1 *N* $HC_2H_3O_2$	0.1 *N* NaOH	...	0.8922	0.8941 ± 0.0006

Discussion

The data of Table I confirm the findings of Furman and Low (*3*) as to the usefulness of the tungsten-nickel system for precise titration of solutions of strong acids, the concentrations of which are 0.1 *N*, but indicate that the system is of little value in titrating 0.01 *N* solutions. Two maxima appear in the graphs of Δ e. m. f./Δ ml. for these titrations. The first, excellent and fairly reproducible, corresponds to the methyl orange end point of the titration and, as pointed out by Furman and Low, represents the neutralization of free alkali and conversion of carbonate to bicarbonate. The second and smaller maximum, corresponding to the phenolphthalein end point, is not always clearly marked in the titration of 0.01 *N* solutions. Its location in graphs for successive titrations varies by as much as 3 per cent, and the acid-base ratios for this maximum in seven titrations show an average deviation of 1 per cent from the mean. The agreement between the mean values for the electrometric and phenolphthalein end-point ratios in the titration of 0.01 *N* sulfuric acid is, therefore, of little significance.

Results of titrations made with the tungsten-silver system are given in Table II. It is evident that this system furnishes a precise method for titrating a strong acid with a strong base in solutions as dilute as 0.001 *N*, and *vice versa* for solutions as dilute as 0.01 *N*. In Figure 1 are shown titration curves typical of those obtained for a single titration with the electrode pairs, tungsten-silver, tungsten-calomel, and silver-calomel. In these graphs *P* marks the point at which phenolphthalein changed color. It will be noted that the potential of the silver electrode passes through a minimum in the neighborhood of this colorimetric end point. At the inflection point the maximum change in potential is in the same direction as that of the tungsten electrode, but comparatively so small that the tungsten-silver system gives a curve with a significant "break" even in titrations of 0.001 *N* solutions. The extent of this break is enhanced by the passage of nitrogen through the solution.

An attempt was made to see whether the tungsten-silver pair permits the precise titration of solutions of strong acids more dilute than 0.001 *N*. To this end 20-ml. samples of approximately 0.01 *N* sulfuric acid and hydrochloric acid, respectively, were added to about 1700 ml. of water, and the titration was made with 0.01 *N* sodium hydroxide. The following data indicate that the use of the tungsten-silver system leads to precise results under the conditions just described:

	End-Point Ratio: Ml. of 0.01 *N* Acid per Ml. of 0.01 *N* Base	
	Electrometric	Colorimetric
H_2SO_4	0.9786 ± 0.001	0.9789
HCl	0.9640 ± 0.0002	0.9656

The data of Table II likewise indicate that this system is suitable for the titration of strong acids with the weak base, ammonium hydroxide, and for the titration of the weak acid, acetic acid. Titration curves for the former are similar to those of Figure 1, with a break not quite so great. Figure 2 is a typical e. m. f.–ml. graph for the titration of acetic acid, in which the first of two inflection points coincides with the phenolphthalein end point, *P*. Graphs for the behavior of the

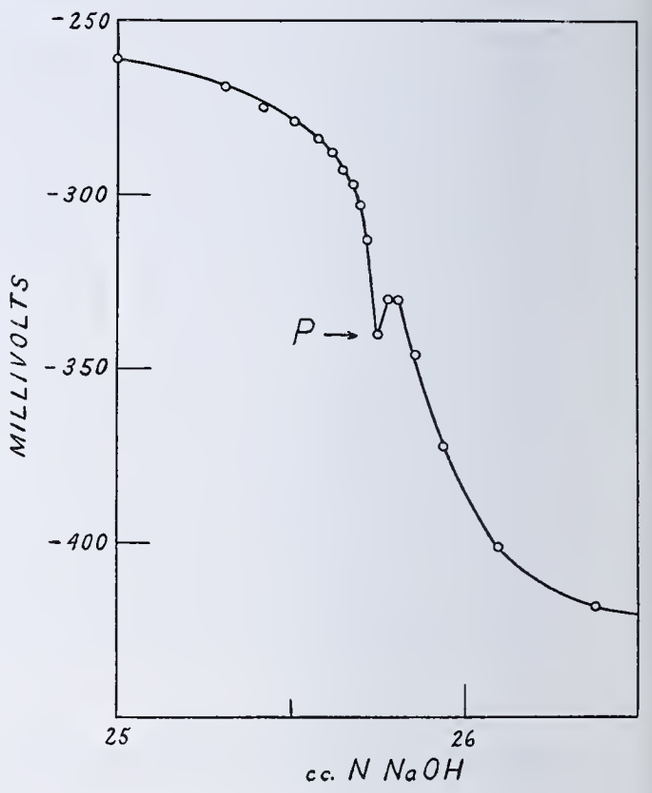

FIGURE 2. TITRATION OF *N* ACETIC ACID
Using tungsten-silver system

silver-calomel and tungsten-calomel pairs during the titration of acetic acid show that to obtain the greatest change in the potential of the silver-calomel system one drop (about 0.03 ml.) more of sodium hydroxide solution is required than for the tungsten-calomel system. Consequently the electrometric end point of the tungsten-silver system arises almost wholly from the behavior of the tungsten electrode; upon addition of base beyond the electrometric end point, changes in the potential of the silver electrode offset those of tungsten to such an extent that two inflection points are observed. Potentiometric readings were not recorded until apparently constant. However, it is possible that in these solutions the electrodes approach equilibrium with extreme slowness and that the behavior observed is due to the system's not having attained equilibrium (*2*). Titration of acetic acid solutions less than 0.1 *N* was not feasible, as the changes in potential were too gradual to permit precise determination of the end point. For the same reason it was not possible in the titra-

Table III. Comparison of End Points

(Solution added: NaOH, approximately 0.01 *N*)

Solution Titrated (Approximate Normality)	End-Point Ratios: Hydrogen-calomel	Tungsten-silver	Phenolphthalein
	Ml. acid per ml. base		
0.01 *N* H_2SO_4	1.022 ± 0.001	1.021 ± 0.002	1.021
0.01 *N* HCl	0.6918 ± 0.0006	0.6926 ± 0.0006	0.6906

tion of sodium carbonate (0.5 *N*) with hydrochloric acid (*N*) to determine precisely the electrical end point corresponding to the phenolphthalein end point. At the methyl orange end point of the carbonate titration, however, the tungsten-silver system gave a definite, reproducible electrometric end point.

To determine the accuracy, as well as the precision, of the tungsten-silver electrode system in titrations of dilute solutions of strong acids, a comparison was made of end-point ratios obtained by the use of hydrogen-calomel, tungsten-silver, and phenolphthalein, respectively. These data appear in Table III.

Summary

The tungsten-nickel electrode system has not been found satisfactory in neutralizations involving dilute solutions. The tungsten-silver system appears to furnish precise and accurate electrical end points in titrations of strong acids by strong bases as dilute as 0.001 *N*, and *vice versa* for 0.01 *N* solutions. In titrations employing more concentrated solutions the system is of value in the neutralization of strong acids by ammonium hydroxide, of acetic acid by a strong base, and of sodium carbonate.

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Extraction and Determination of Pyrethrin I in Ground Pyrethrum Flowers

An Improved Apparatus

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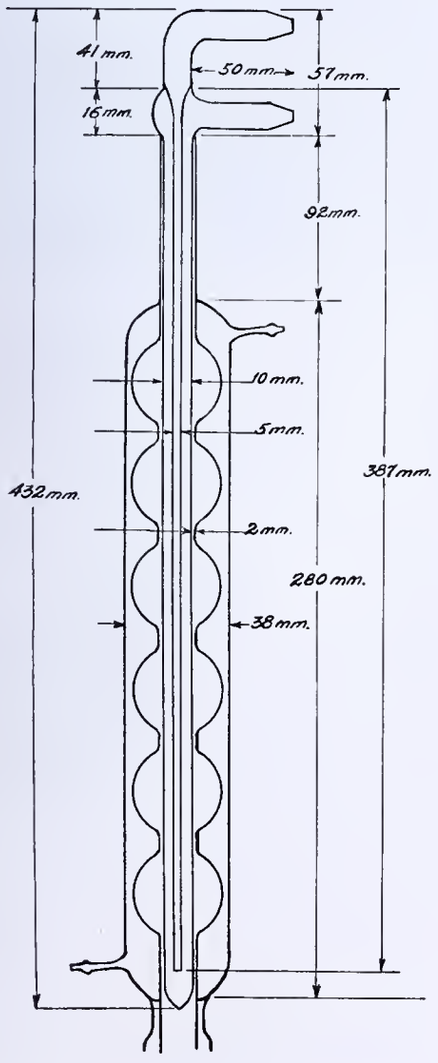

Figure 1. Auxiliary Condenser

MANY of the more reliable methods (*1–4*) developed in recent years for the extraction of pyrethrum flowers for analysis recommend the use of low-boiling-point petroleum ether (20° to 40° C.) as a solvent, because it can completely remove the pyrethrins without removing other substances which will interfere with the method subsequently employed for the pyrethrin determination, and this solvent may itself be easily removed with the least amount of heat, so that there is little chance of loss of the pyrethrins.

A simple and inexpensive modification of an apparatus for the extraction and determination of pyrethrin I in ground pyrethrum flowers is proposed. The objects of the changes are primarily to reduce the loss of petroleum ether used for extraction and to eliminate transfer of the extracted and subsequently refluxed solution to a larger container for alcohol removal. Modifications of the condenser amount to insertion of a cold-water column into each condenser and use, as a receiving flask, of a 500-cc. Kjeldahl flask with the same size of interchangeable ground-glass joints as in the rest of the apparatus. Frothing during the evaporation of alcohol is reduced and less attention is required.

The use of a Soxhlet with either an Allihn or a Graham condenser, all with ground-glass joints, in a 7- or 8-hour extraction of ground pyrethrum flowers with low-boiling-point petroleum ether has heretofore resulted in a 60 to 75 per cent loss of a 150-cc. volume of solvent used, especially during warm weather (29.44° C., 85° F., or above) or whenever the temperature of water used for circulating in condensers is much above 25° C. This has been due chiefly to inefficient or insufficient cooling surface in the condenser rather than to leakage in glass connections.

To remedy the difficulty without increasing the length of the condenser so as to make the height of the apparatus too great for convenient handling, a double tube equivalent in length (30 to 42.5 cm., 12 to 17 inches) to the Allihn condenser, and of a diameter to allow a clearance of 2 to 3 mm. at the bulb constrictions, was constructed (Figure 1). It can be inserted into the condenser to the bulblike enlargement that acts as a rest point, and then

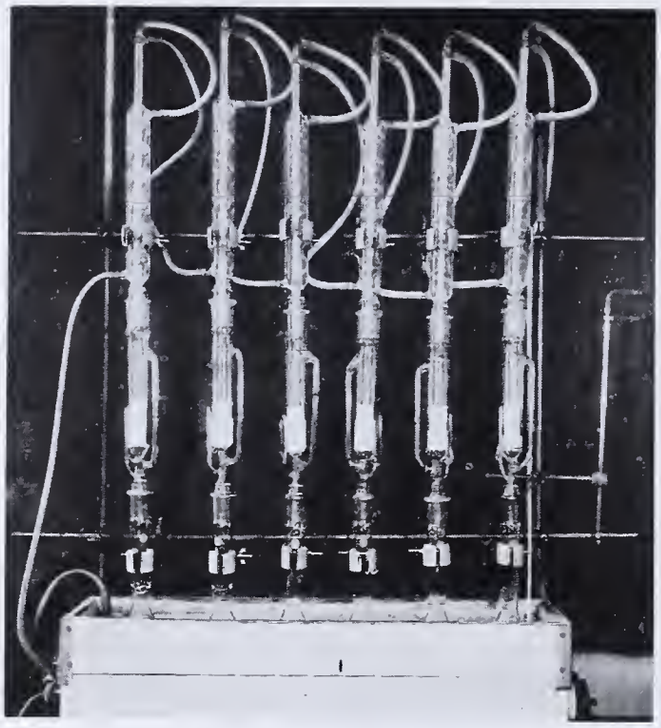

FIGURE 2. SIX EXTRACTORS IN OPERATION WITH CONDENSER MODIFICATIONS
Single unattached condenser modifier at extreme right

connected to receive the outflow of cold circulating water from the Allihn condenser before it goes into the next condenser. This modification has the effect of a double condenser, or a condenser within a condenser.

According to several published analytical methods (*1–4*), the extracted material after complete removal of the solvent must be refluxed with 0.5 *N* alcoholic sodium hydroxide and then, with about 100 cc. of water, transferred to a container (500- to 800-cc. beaker) large enough to accommodate frothing, which always takes place during the removal of alcohol with heat and requires much attention to prevent loss of sample.

If a 500-cc. Kjeldahl flask, specially made with the same size of ground-glass joint as that of the Soxhlet and Allihn condenser, is substituted for a receiving flask in the initial extraction, refluxing and subsequent removal of the alcohol after the addition of water may be accomplished without any transfer, by simply heating the flask with a very low flame in a Kjeldahl digestion rack. Frothing will still exist in the first minutes of boiling and requires some attention, but this soon diminishes as the removal continues, accompanied by a decrease in volume.

Figure 2 shows six complete extractors with the above-mentioned modification. Each Kjeldahl flask used as a receiver is immersed in a water bath (45° to 50° C.) resting on an electric hot plate, with a slow continuous stream of cold water flowing into the bath to replenish the loss of water due to evaporation and at the same time prevent the temperature of the bath from rising very much above the desired range.

Using the modified apparatus, the loss of ether and volume required for extraction have been reduced approximately 50 and 33 per cent, respectively. In addition, little attention is required during the removal of alcohol by heat. These modifications have resulted in greater economy, considerable saving of time, and elimination of a health hazard in the excessive escape and possible accumulation of fumes of a highly volatile solvent.

Acknowledgment

The author is indebted to R. McCready for the suggestions and construction of the type of condenser modifier used.

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New Photoelectric Fluorimeter and Some Applications

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FLUORIMETRIC methods of quantitative analysis have increased in popularity in recent years. These methods must be used when the substances to be determined are present in such small amounts that colorimetric methods cannot be used, but are limited to substances which fluoresce when irradiated by light of the proper wave lengths, usually between 3000 and 4500 Å.

Fluorimeters of two general types are in use. One type of instrument depends on a visual comparison of an unknown with a standard of fluorescence. When the unaided eye is used to determine the match of unknown and standard, the sensitivity of the method is low and decreases with increasing concentration. The use of an instrument such as the modified colorimeter of Josephy (*7*) or a Pulfrich photometer (*12*) increases sensitivity and accuracy of match over those possible by the unaided eye.

The other type of instrument employs a photocell and galvanometer to measure the fluorescence. Most European workers use the Cohen (*1*) fluorimeter, a simple instrument with a test tube to hold the fluorescing solution. The fluorescence is measured by a barrier-layer photoelectric cell and a high-sensitivity galvanometer which can be used only at one tenth to one fiftieth the full sensitivity because of fluctuations of lamp intensity (*4*). In the Hennessy and Cerecedo (*3*) modification, a cuvette with rectangular faces holds the fluorescing solution; and, apparently, the galvanometer can be used at full sensitivity. Hand (*2*), however, uses an instrument in which a relatively insensitive pointer type of galvanometer is the measuring instrument. To obtain consistent results with these instruments, the lamp intensity must remain constant long enough for a measurement to be made of the unknown and then of the standard.

By measuring the fluorescence of an unknown solution in terms of a standard of fluorescence, galvanometer drifts and sudden movements caused by changes in lamp intensity can be eliminated. If the ratio of the fluorescence of an unknown

to that of a standard be measured by a two-photocell balanced-circuit, a high-sensitivity galvanometer can be used at full sensitivity as a null-point indicator; and the range of concentrations measurable can be changed by changing the concentration of the standard. An instrument (made by the Klett Mfg. Co., New York, N. Y.) which incorporated these features was designed and has been used for a variety of measurements for 2 years in this laboratory. Since no other fluorimeter, as yet, employs these principles of design, it seems desirable to describe this one and to indicate how it has performed in some of the quantitative procedures in which it has been tested.

The Fluorimeter

The design of the instrument is such that the potentiometer reading is proportional to the intensity of the fluorescent light emitted by the unknown solution.

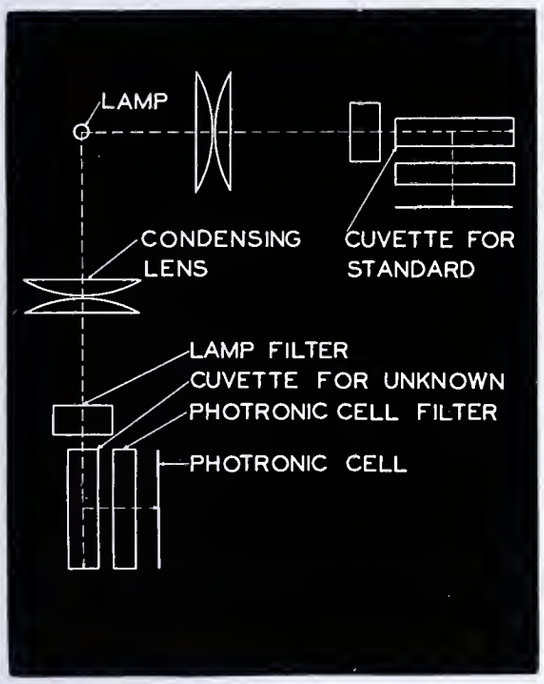

FIGURE 1. ARRANGEMENT OF OPTICAL PARTS OF FLUORIMETER

Ultraviolet or other lines from a type H-4 mercury lamp excite the fluorescence of the solutions in the cuvettes (Figure 1). The lines are isolated by glass filters (the lamp filters). The fluorescent light passes through a face of the cuvette that is parallel to the ultraviolet light beam, then through another light filter (the photocell filter) to a barrier-layer photocell. The two cuvettes are irradiated by the same lamp at the same time; one holds the unknown solution; the other, a standard solution, usually quinine sulfate. The photocells are arranged in a compensated circuit, so that fluctuations in lamp intensity do not affect the operation of the instrument. The ratio of the fluorescence of the unknown to that of the standard is indicated by a built-in potentiometer with a 300-division linear scale.

A portable mirror-type galvanometer with a sensitivity of 0.004 microampere per division is sufficiently sensitive as a null-point indicator for most measurements. All the work reported here was done with a Leeds & Northrup Type R galvanometer used at a sensitivity of 0.001 microampere per millimeter. By making the measurements in terms of a standard, all uncertainties caused by fluctuations and drifts of lamp intensity, nonlinear galvanometer scale, and inconstancy of galvanometer response are eliminated. (Summerson, *9*, gives details concerning the advantages of the two-cell balanced circuit.)

The standard must fluoresce more than the unknown. Because doubling the concentration of the standard halves the potentiometer reading for a particular unknown solution, the range and sensitivity of the instrument can be changed by changing the concentration of the standard. Quinine sulfate U. S. P., 1 or 2.5 mg. per liter in 0.1 *N* sulfuric acid, most often served as a standard solution, although glass standards could have been used.

Filters for a particular determination must be selected with reference to the absorption bands of the fluorescent substance, the concentration range to be measured, and the wave-length limits of the light emitted by the fluorescing solution. The lamp filter must pass lines that are absorbed by the substance which is to fluoresce. For the determination of small amounts of material, the lines that excite the fluorescence should be as near as possible to absorption peaks of the absorption spectrum of the fluorescing substance. To obtain a low and reproducible blank, the photocell filter must absorb those lines of the mercury arc which are passed by the lamp-filter. When this condition is satisfied, a small amount of suspended material in the solution in the cuvette will not affect the response of the instrument.

The determination of some substances can be made more specific by selecting a photocell filter that transmits a band of light not much wider than the band of fluorescent light. For example, the Corning 306 filter can be used in determining thiochrome; but a 306 plus a 430 filter would give a more specific response to thiochrome if other substances which gave fluorescent light of wave lengths longer than about 5000 Å. were present. The filters are all Corning Glass Works polished glass filters and are designated by their Corning numbers. The filters are not necessarily of standard thickness, but the thickness is given for each filter.

By assuming that the intensity of the fluorescent light is proportional to the amount of the exciting light absorbed by the unknown solution and that Beer's law holds for the absorption, the relation between the potentiometer reading, P, and the characteristics of the fluorescing substance is given by

$$P = KI(1 - 10^{-\alpha c b}) = KIf \qquad (1)$$

in which I is the intensity of the exciting line at the lamp end of the cuvette, α is the specific absorption coefficient of the substance for the exciting line, c is the concentration of the fluorescing substance, and b is the length (36 mm.) of the opening in the mask over the photocell. The dimensions of the mask determine the volume of the solution from which the fluorescent light comes and remove the necessity for using accurately measured volumes of solution in the cuvettes. Constant K includes, among other factors, the dimensions of the light beam, the area of the mask opening, the transmission band of the photocell filter, and the spectral sensitivity of the photocell. For a particular instrument, and

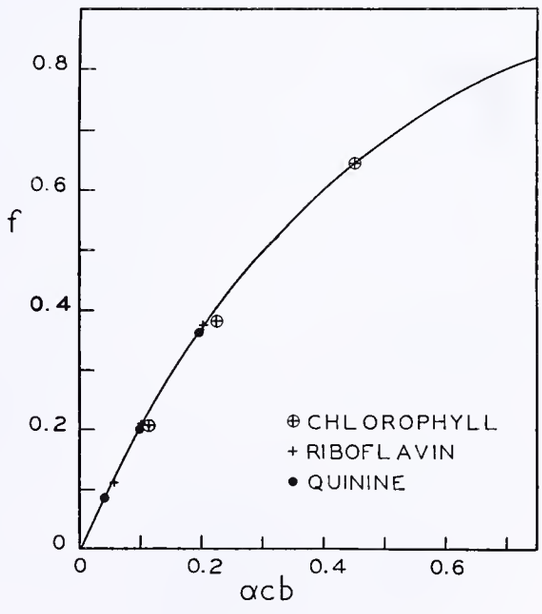

FIGURE 2. f AS A FUNCTION OF αcb (EQUATION 1)
Fluorescence of chlorophyll excited by 4358 Å. line, of quinine by the 3660 Å. lines, and of riboflavin by 4358 Å. line

a given combination of lamp and photocell filters, standard, and substance to be determined, the variables are P and c.

The relation between P and αcb was tested by measuring solutions of quinine, riboflavin, and ethyl chlorophyllide for small and large values of αcb by varying both c and the value of α (by changing the wave length of the exciting line). The results are given in Figure 2, in which the measurements are indicated by the points and the curves are the graph of f against αcb. The points are located in the following manner: The αcb is calculated for each concentration of a series of measurements and a value of f for each concentration is calculated from

$$f = \frac{P}{P_m} f_m \qquad (2)$$

where P_m is the potentiometer reading and f_m is the value of f for the highest concentration. The agreement between the calculated and measured values of f is good for a wide range of concentrations. The concentration, c, is expressed as grams per liter and $\alpha = \frac{\epsilon}{\text{mol. wt.}}$ = specific absorption coefficient.

$\alpha cb = \epsilon Cb$, where ϵ = molecular absorption coefficient and C = moles per liter; b is expressed as cm.

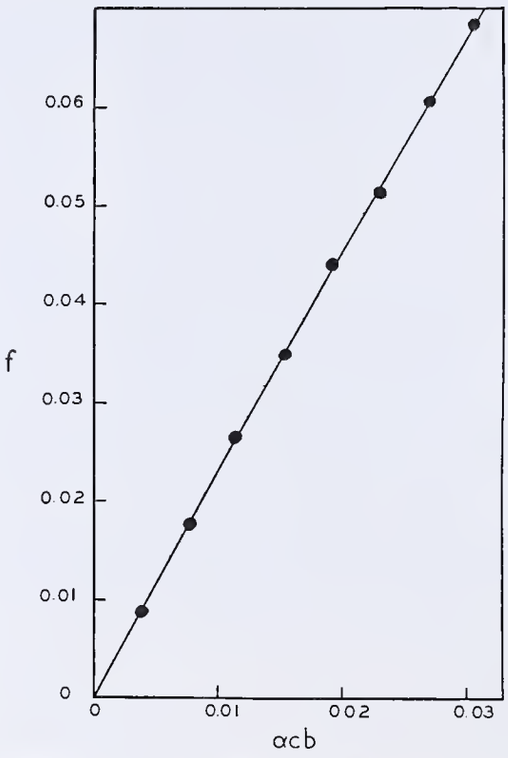

FIGURE 3. f AS A FUNCTION OF αcb (EQUATION 1) WHEN αcb IS SMALL

Fluorescence of the quinine excited by 3660 Å. lines. Potentiometer reading for largest αcb (0.8 mg. of quinine sulfate per liter) 266

In some measurements—quinine and thiochrome, for example—large values of αcb are not usually measured. Is the agreement as good for small values of αcb as for large values, and is there a range of concentrations through which the relation between P and αcb is linear? The calculated curve and measured points for a series of concentrations of quinine sulfate from 0.1 to 0.8 mg. per liter in 0.1 N sulfuric acid are given in Figure 3. The line is straight for values of αcb less than 0.02 (0.5 mg. of quinine sulfate per liter) and slightly curved for values up to 0.032, which is the largest that can be measured with the 1 mg. per liter quinine standard and the particular lamp and photocell filters used.

Whether the potentiometer-concentration curve will be nearly straight or strongly curved depends upon the value of α, the intensity of the exciting lines, the fraction of the absorbed light emitted as fluorescent light, and the spectral sensitivity of the photocell. (The intensity, the wave length of the exciting line, and the spectral sensitivity of the photocell do not have to be the same for the unknown and for the standard.) The concentration range that can be measured for a particular set of conditions always lies between zero and the concentration that gives a potentiometer reading of 300. If the measurable concentration range is large, the potentiometer scale reading–concentration curve will be strongly curved; if the range is short, the potentiometer–concentration curve will approach a straight line.

TABLE I. RANGE OF CONCENTRATIONS OF SUBSTANCES DETERMINED WITH FLUORIMETER

("Lower" concentration gives potentiometer reading of approximately 10 with filters and standard indicated. Filters for quinine standard were lamp, 597; photocell, 306.)

Substance Determined	Concentration		Filters		Quinine Standard
	Lower	Highest	Lamp	Photocell	
	Mg. per liter				*Mg. per liter*
Aluminum	0.04	0.9	554	338	1
	0.1	6	597	306	1
Chlorophyll	0.03	0.6	585	243	1
	0.1	5	597	243	1
Quinine	0.03	0.9	597	306	1
Riboflavin	0.005	0.15	554	351	0.25
	0.02	0.6	554	351	1
	0.05	2	597	351	2.5
Thiochrome	0.005	0.1	597	306	1

Analyses with the Fluorimeter

Methods for determining thiamin, riboflavin, chlorophyll, and aluminum have been tested with this fluorimeter to determine the possibilities of the instrument and the limitations of the methods. The solutions did not necessarily contain accurately measured quantities of the substances and no attempt was made to establish standard curves. The concentration of the standards, the filters, the upper concentration of the unknown, and the concentration for which there is a potentiometer reading of 10 are shown in Table I.

In all the work reported here, the lamp filter for the standard solutions was a 597 (5.25-mm.) and the photocell filter was a 306 (3.2-mm.).

THIAMIN. Pure thiochrome was dissolved in *n*-butanol. The first test with this compensated circuit fluorimeter showed that thiochrome decomposed rapidly when irradiated by 3660 Å. lines and was about half destroyed in 10 minutes. By using the following procedure accurate measurements were possible:

With the quinine standard in place, put the cuvette containing the thiochrome into the instrument and start a stop watch the moment the cuvette enters the beam of ultraviolet light; at definite intervals after placing the cuvette in the light beam, measure the fluorescence, at least four times in the first 2 minutes; correct the measurements for the fluorescence of the blank; plot the logarithm of the corrected potentiometer readings against time on semilog paper; and extrapolate to zero time to obtain the potentiometer reading of the undecomposed thiochrome. These potentiometer readings at zero time for solutions of different concentrations plotted against the concentration gave a straight line. If an error of 1 or 2 per cent is of no importance, read the potentiometer as quickly as possible and assume that no decomposition has occurred. The upper limit of measurement for the thiochrome method for thiamin with this fluorimeter is about 150 micrograms of thiamin per liter; the lower limit, about 1 microgram per liter.

Caution. Butanol can remove enough grease from an apparently clean stopcock of a separatory funnel to give an appreciable fluorescence, and more fluorescing substance from a Whatman No. 42 filter paper used to remove suspended material than is contained in some solutions of thiochrome.

RIBOFLAVIN. Riboflavin can be determined either fluorimetrically or colorimetrically. Supplee, Bender, and Jensen (*10*) compared visually the fluorescence of an unknown with a series of standards containing known amounts of riboflavin. Josephy (*7*), using a modified Duboscq-type colorimeter with sodium fluorescein as a standard, could determine amounts as small as 0.3 microgram in a 2-ml. sample. The methods using photoelectric fluorimeters for determining the fluorescence of

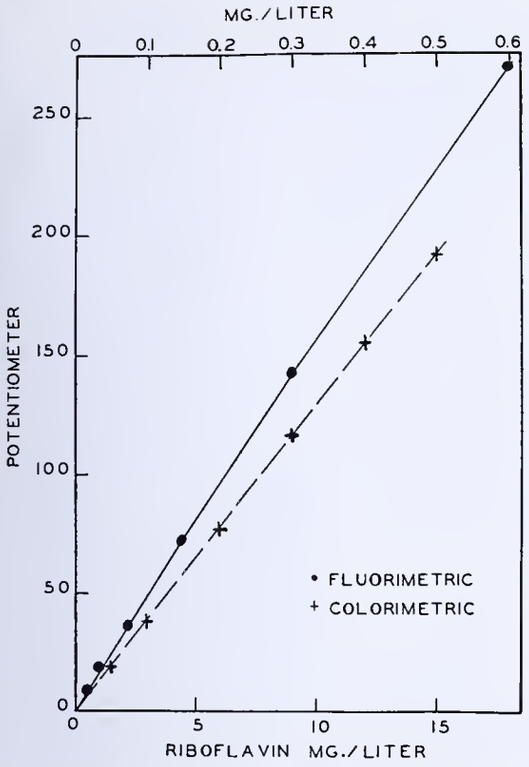

FIGURE 4. COMPARISON OF CALIBRATION CURVES FOR FLUORIMETER AND COLORIMETER IN DETERMINATION OF RIBOFLAVIN

Lower curve and scale of concentrations are for colorimeter (42 filter). Upper curve and scale at top are for fluorimeter using a 554 lamp filter and a 351 photocell filter

)oflavin are more accurate and sensitive than the visual methods d the results can be corrected for the presence of nonriboflavin orescing and light-absorbing substances as Hodson and Norris) have done.

This fluorimeter and a Klett-Summerson colorimeter (*9*) uipped with 42 filter have been compared over a wide range of ncentrations of riboflavin dissolved in 66 per cent acetone. ıe colorimeter was satisfactory for concentrations between 1 d 20 mg. of riboflavin per liter (Figure 4). The fluorimeter uld be used for concentrations between 0.005 and 2 mg. per er (Table I). By the selection of various filters and quinine ındards, several ranges of concentrations could be determined th the fluorimeter. Measurements could be made by the orimetric method on solutions too dilute to give any response th the colorimeter. Not only did the fluorimeter determine ıaller amounts of riboflavin than the colorimeter, but small ıounts of suspended material did not interfere with the deternations as they did with the colorimetric determinations.

The riboflavin, or similar substance, produced by several fungi solutions in which they had grown, was measured by the ethod of Hodson and Norris (*5*). The results will be pubhed elsewhere.

The corrections of Hodson and Norris for light-absorbing and nriboflavin fluorescing substances are most easily applied when e potentiometer readings are proportional to the concentration riboflavin. This condition was satisfied when a 554 (5.2-m.) lamp filter was used at concentrations below 0.3 mg. of)oflavin per liter (Figure 4).

CHLOROPHYLL. The fluorescence of chlorophyll has been ed in characterizing it for many years. Zscheile (*13*) found)st of the fluorescence of an ether solution of chlorophyll *a* d of chlorophyll *b* to be between 6200 and 7600 Å. This orescence can be used to determine very small quantities of lorophyll.

A series of dilutions of an unknown mixture of crystalline ethyl lorophyllide *a* and *b* prepared by F. M. Schertz was made in butanol and used to determine a calibration curve for the orimeter. A 243 (3.3-mm.) filter, which cuts off wave lengths s than 6100 Å. and transmits freely above 6300 Å., was placed er the photocell that measured the fluorescence of the chloroyll. Results for two lamp filters are given (Table I). The 5 (1.5-mm.) filter transmits the violet and ultraviolet lines of e mercury arc with little absorption. The lines passed by this ter excite the fluorescence of chlorophyll to such an extent that the highest concentration that can be determined with the 1 mg. per liter standard is low (0.6 mg. per liter, Table I); and, consequently, the calibration curve is straight. The 597 (5.25-mm.) lamp filter which passes only the ultraviolet lines excites much less fluorescence than the 585 filter, with the result that the highest concentration that can be determined is 5 mg. per liter; and the calibration curve is strongly curved. The thermocouple photometer method of Johnston and Weintraub (*6*) has a sensitivity almost as high as the fluorimetric method but is more difficult to use and requires more time for its operation. Both the colorimeteric and fluorimetric methods for total chlorophyll probably give different results for different ratios of chlorophyll *a* and *b* for the same total concentration.

If the chlorophyll solution is treated with dilute sulfuric acid, there is a rapid decrease in fluorescence to about 70 per cent of the original value as the result of conversion of chlorophyll into pheophytins. In quantitative work, the precautions recommended by Mackinney (*8*) must be observed.

To demonstrate the value of the fluorimetric method, the chlorophyll content of one *Lemna minor* was determined. The plant (fresh weight 2 mg.) was ground with a few milliliters of *n*-butanol, diluted to 20 ml. with *n*-butanol, and the fluorescence determined. The potentiometer reading was 39. Reference to the appropriate calibration curve indicated that the plant contained 6 micrograms of chlorophyll.

ALUMINUM. White (*11*) has given a review of recent work on fluorimetric methods applied to inorganic analysis. As an example of the application of the instrument to inorganic analysis, a calibration curve was made for the morin method of determining aluminum. The method was slightly different from the one used by White and Lowe (*12*).

To the aluminum in an acetate buffer at pH 4, 1 ml. of aqueous morin solution (0.75 gram per liter of morin, Eastman) was added, and the sample was diluted to 25 ml. with the acetate buffer solution. The results are given in Table I. The calibration curve for the filter combination 597-306 was straight up to 0.6 mg. per liter and then curved toward the concentration axis for higher concentrations. The filter combination 554-338 gives a linear calibration curve to 0.3 mg. per liter and a slightly curved one for higher concentrations. The former filter combination gives about five times the range and the latter filter combination gives at least three times the sensitivity of the instrument of White and Lowe. No effort was made to obtain conditions of maximum sensitivity.

Other Uses for Fluorimeter

The fluorimeter is provided with two photocells, one in each light beam, which can be connected to the potentiometer to form a Klett-Summerson type of colorimeter. For this reason, the potentiometer has the logarithmic scale of that colorimeter in addition to the linear scale of the fluorimeter. The colorimeter can be used with the lines in the visible spectrum and the 3660 lines of the mercury arc which can be isolated by glass filters, or the mercury lamp can be replaced by an incandescent lamp to use the instrument as a colorimeter of the usual type with filters that isolate narrow spectral regions. The colorimeter has the same linear calibration curve for substances that follow Beer's law and the ease of correction for blanks that is characteristic of the Klett-Summerson colorimeter.

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Hydrometer for Turpentine Indicating Pounds per Gallon

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IN COMMERCE turpentine is measured by both volume and weight. Domestic transactions are based on units of the standard gallon and the avoirdupois pound; and transportation charges are computed from weight. A lot of turpentine may be subjected to a considerable variation in temperature. While its weight will be affected but slightly by such changes, its volume will vary with the temperature in accordance with its coefficient of expansion.

The weight of a known volume of water and other liquids of unvarying composition at a definite temperature, or the volume of a known weight when facilities for actual determinations are not available, can be calculated from standard data. For turpentine and other liquids whose composition and properties vary it is necessary to determine the weight of a unit volume before such calculations can be made.

Federal specification LLL-T-791a requires that turpentine for use by the Government be purchased by volume, the unit being a gallon of 231 cubic inches at 60° F., or by weight in units of pounds or hundred pounds, and gives directions for correcting gallonage to the standard temperature of 60° F. and for determining the weight of a gallon in pounds. A gallon of acceptable turpentine must weigh from 7.16 to 7.29 pounds at 60° F. In making volume corrections a coefficient of 0.000945 per degree Centigrade, or 0.000525 per degree Fahrenheit, is used. The specific gravity of a sample is determined at 15.5° C./15.5° C. by any method that is accurate within two points in the fourth decimal place, and the weight of a gallon in pounds is determined by multiplying the figure thus obtained by 8.33.

Tables are available (*1*, *3*, *4*) for converting observed specific gravity and Baumé readings taken at other temperatures to true values at 60° F. and also tables showing the pounds per gallon corresponding to various degrees Baumé and specific gravities. The change in density in grams per milliliter of turpentine from 10° to 40° C. is given (*1*) as well as the change in weight in pounds of a gallon of turpentine from 20° to 110° F. (*4*).

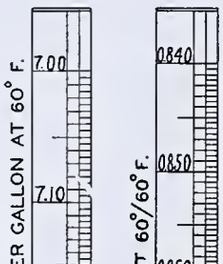


FIGURE 1

Specific gravity may be determined by the pycnometer or weighing bottle, the Westphal balance and the hydrometer. Baumé gravity is determined with the Baumé hydrometer. Because of low cost, portability, and simplicity of operation hydrometers commonly are used for turpentine, and properly used will give results of accuracy commensurate with that obtained in commercial weighing and gaging of liquids.

A method for weight-volume conversion of turpentine which will eliminate or reduce the use of tables is obviously desirable. A hydrometer indicating weight in air in pounds per gallon at 60° F. was not available, although hydrometers for reading per cent of alcohol and sugar and other special scales are in common use. An instrument manufacturer constructed four experimental hydrometers having 5-inch scales graduated as shown in Figure 1; over-all length, 12 inches; diameter of float chamber, 1 inch; and length of stem, 7 inches. These instruments, on standardization by the

TABLE I. WEIGHT IN AIR IN POUNDS PER GALLON OF TURPENTINE FROM 32° TO 95° F.

Temperature of Reading, ° F.														
32	7.261	7.271	7.281	7.290	7.300	7.310	7.320	7.330	7.340	7.350	7.360	7.369	7.379	7.389
34	7.254	7.264	7.274	7.283	7.293	7.303	7.313	7.323	7.333	7.343	7.353	7.562	7.372	7.382
36	7.247	7.257	7.267	7.276	7.286	7.296	7.306	7.316	7.325	7.335	7.345	7.365	7.375	7.385
38	7.249	7.239	7.249	7.259	7.269	7.279	7.289	7.299	7.308	7.318	7.328	7.338	7.348	7.358
40	7.232	7.242	7.252	7.262	7.272	7.282	7.292	7.302	7.311	7.321	7.331	7.341	7.351	7.361
42	7.225	7.235	7.245	7.255	7.265	7.274	7.284	7.294	7.304	7.314	7.324	7.334	7.344	7.354
44	7.218	7.228	7.238	7.247	7.257	7.267	7.277	7.287	7.297	7.307	7.317	7.327	7.337	7.347
46	7.210	7.220	7.230	7.240	7.250	7.260	7.270	7.280	7.290	7.300	7.310	7.319	7.329	7.339
48	7.203	7.213	7.223	7.233	7.243	7.253	7.263	7.273	7.283	7.293	7.303	7.302	7.322	7.332
50	7.196	7.206	7.216	7.226	7.236	7.246	7.256	7.266	7.276	7.286	7.296	7.305	7.315	7.325
52	7.189	7.199	7.209	7.219	7.229	7.239	7.249	7.259	7.269	7.278	7.288	7.298	7.308	7.318
54	7.182	7.192	7.202	7.211	7.221	7.231	7.241	7.251	7.261	7.271	7.281	7.291	7.301	7.311
56	7.174	7.184	7.194	7.204	7.214	7.224	7.234	7.244	7.254	7.264	7.274	7.284	7.294	7.304
58	7.167	7.177	7.187	7.197	7.207	7.217	7.227	7.237	7.247	7.257	7.267	7.277	7.287	7.297
60	7.160	7.170	7.180	7.190	7.200	7.210	7.220	7.230	7.240	7.250	7.260	7.270	7.280	7.290
62	7.152	7.162	7.172	7.182	7.192	7.202	7.212	7.222	7.232	7.242	7.252	7.26	7.272	7.282
64	7.145	7.155	7.165	7.175	7.185	7.195	7.205	7.215	7.225	7.235	7.245	7.25	7.265	7.275
66	7.137	7.147	7.157	7.167	7.177	7.187	7.197	7.207	7.217	7.227	7.237	7.247	7.257	7.267
68	7.130	7.140	7.150	7.160	7.170	7.180	7.190	7.200	7.210	7.220	7.230	7.240	7.250	7.260
70	7.122	7.132	7.142	7.152	7.162	7.172	7.182	7.192	7.202	7.212	7.223	7.233	7.243	7.253
72	7.114	7.124	7.134	7.144	7.154	7.164	7.174	7.185	7.195	7.205	7.215	7.225	7.235	7.245
74	7.107	7.117	7.127	7.137	7.147	7.157	7.167	7.178	7.188	7.198	7.208	7.218	7.228	7.238
76	7.099	7.109	7.119	7.129	7.139	7.149	7.159	7.170	7.180	7.190	7.200	7.210	7.220	7.230
78	7.092	7.102	7.112	7.122	7.132	7.142	7.152	7.162	7.172	7.183	7.193	7.203	7.213	7.223
80	7.084	7.094	7.104	7.114	7.124	7.134	7.144	7.155	7.165	7.175	7.185	7.195	7.205	7.215
82	7.076	7.086	7.096	7.106	7.116	7.127	7.137	7.147	7.157	7.167	7.177	7.188	7.198	7.208
84	7.069	7.079	7.089	7.099	7.109	7.119	7.129	7.140	7.150	7.160	7.170	7.180	7.191	7.201
86	7.061	7.071	7.081	7.092	7.102	7.112	7.122	7.132	7.142	7.152	7.162	7.173	7.183	7.193
88	7.054	7.064	7.074	7.084	7.094	7.104	7.114	7.125	7.135	7.145	7.155	7.165	7.175	7.186
90	7.046	7.056	7.066	7.076	7.087	7.097	7.107	7.117	7.127	7.137	7.148	7.158	7.168	7.178
92	7.039	7.049	7.059	7.069	7.079	7.089	7.099	7.109	7.120	7.130	7.140	7.150	7.160	7.171
94	7.031	7.041	7.051	7.061	7.071	7.082	7.092	7.102	7.112	7.122	7.132	7.143	7.153	7.163
95	7.027	7.037	7.047	7.058	7.068	7.078	7.088	7.098	7.108	7.119	7.129	7.139	7.149	7.159

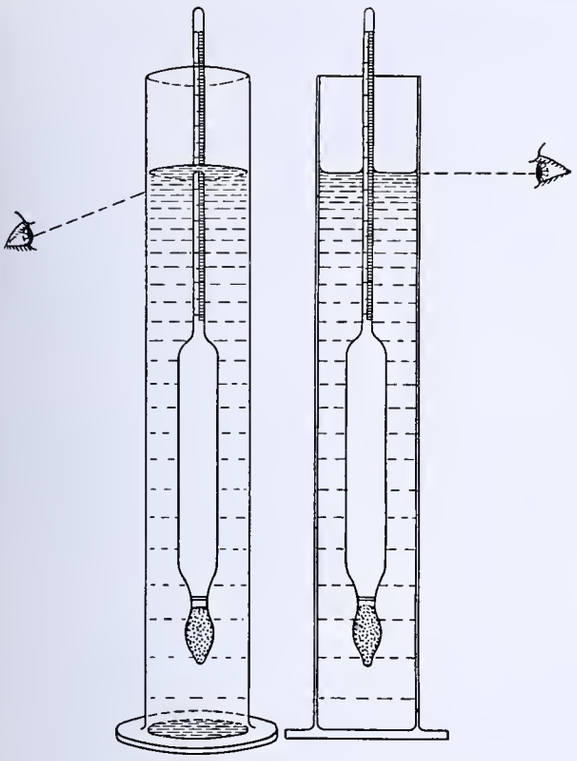


FIGURE 2. READING HYDROMETERS

Jational Bureau of Standards, were found to require maxi-
ıum scale corrections of 0.002. The comparison liquid used
ı the standardization was mineral oil, which has a surface
ension very close to that of turpentine. The coefficient of
ubical expansion of the glass used in the
ıstruments was stated by the manufac-
urer to be 0.000027 per degree Centigrade,
r 0.000015 per degree Fahrenheit.

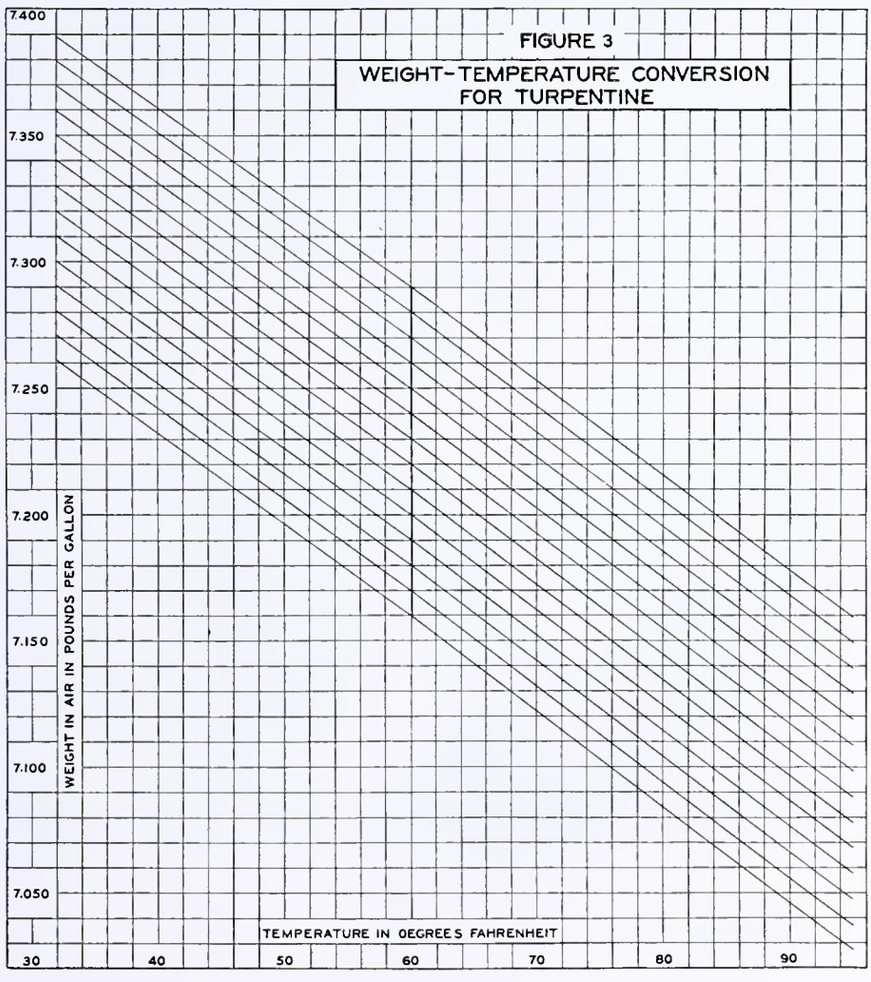
The difference between the observed
eading and correct value is approxi-
ıately 0.001 pound per gallon for each
0° F. above or below 60° F., subtracted
rom observed readings made above
0° F. and added to those made below
his temperature. A sample on which a
ydrometer reading at 80° F. showed
.150 pounds per gallon has a true weight
ı air at 80° F. of 7.148 pounds per gallon.
uch small corrections generally need not
e applied, since the limit of accuracy in
eading the hydrometer is ±0.001, and
change of only 0.3° F. in the sample
ill result in a change of 0.001 in hydrom-
ter reading.

Table I and Figure 3 are designed pri-
ıarily to convert observed hydrometer
eadings at other temperatures to pounds
er gallon at 60° F. They may be used,
ith corrections above noted, for deter-
ıining change in weight of a gallon of
urpentine with temperature. The data
ısed as a guide in preparing Table I (*3*)
ere obtained experimentally from 14 sam-
les of gum spirits of turpentine and
wo samples of steam-distilled wood tur-
entine using a specific gravity hydrom-
ter made of the same type of glass used
n the pounds per gallon hydrometer and
onstructed by the same manufacturer.

To obtain the true weight in air of turpentine at the standard temperature of 60° F. from observed readings taken at other temperatures on a pounds per gallon hydrometer standardized for 60° F., locate observed reading, by interpolation if necessary, in horizontal line opposite temperature at which reading was made, and follow in vertical column to corresponding reading at 60°, which is the true weight in air at 60°.

The thermal expansion of destructively distilled or wood spirits of turpentine has been indicated (*1*) as about 10 per cent greater than that of either gum spirits of turpentine or steam-distilled wood turpentine. It is probable that the material produced at that date (1911) differed markedly from present-day destructively distilled wood turpentine. The writer has found aromatic hydrocarbons in very old destructively distilled wood turpentine, the presence of which would increase the thermal expansion of the turpentine. No data have been found on the thermal expansion of sulfate wood turpentine. In order to determine the relative thermal expansions of the four kinds of turpentine and to test the new hydrometer, three samples of sulfate wood, three of destructively distilled wood, two of steam-distilled wood, and two of gum spirits representing current production were obtained. Readings on both the specific gravity and the pounds per gallon hydrometer were taken on each sample at 34°, 68°, and 95° F. These tests showed that thermal expansion of turpentine is independent of kind. Therefore, the tables for specific and Baumé gravity hydrometers (*3*), and Table I and Figure 3 may be used for any of the four kinds of turpentine.

The accuracy of a determination will depend on the accuracy of the hydrometer and thermometer and the care with which they are used. To obtain the highest degree of accuracy, both the hydrometer and the thermometer should be standardized by the National Bureau of Standards.



Method of Reading Hydrometers

Fill a clear glass jar or cylinder having a height equal to the length of the hydrometer and an inside diameter at least 1 inch greater than the diameter of the hydrometer bulb to within about 2 inches of the top with the turpentine to be tested; place a thermometer in the jar, and set it on a table in a sheltered place. Carefully immerse the hydrometer in the turpentine to a point slightly below that to which it naturally sinks and then allow it to float freely. Be sure the hydrometer is not in contact with the jar or the thermometer. When the temperature as registered by the thermometer has become stationary and the turpentine and the hydrometer are free from air bubbles and are at rest, place the eye slightly below the plane of the surface of the turpentine (Figure 2, left) and raise the eye slowly until this surface seen as an ellipse appears to be a straight line (Figure 2, right). Take the reading of the instrument at the point at which this line cuts the hydrometer scale (*2*). The third decimal on the scale must be determined by interpolating (estimating) from the smallest division on the scale. Record the reading of the hydrometer and the thermometer (first making corrections if instruments have been standardized). If the temperature of the turpentine is not 60°, the weight per gallon at 60° and other temperatures may be obtained from Table I or Figure 3.

An enlargement of Figure 3 on millimeter cross-section pape may be obtained by writing the Naval Stores Research Division Bureau of Agricultural Chemistry and Engineering, Washing ton, D. C.

While hydrometers indicating pounds per gallon are no at present listed by instrument makers and apparatus supply firms, a number of instrument makers have indicated tha they can furnish instruments suitable for use with turpentine

This type of hydrometer of suitable range with accom panying tables or graphs should be useful for the weight volume conversion of mineral oils and other liquids.

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(1) Bearce, H. W., Bur. Standards, *Tech. Paper* 9 (April, 1912).
(2) Natl. Bur. Standards, "National Standard Petroleum Oil Tables" *Circ.* C410, p. 4 (March 4, 1936).
(3) Smith, W. C., and Veitch, F. P., U. S. Dept. Agr., *Circ.* 110 (Feb. 1930).
(4) Veitch, F. P., and Grotlisch, V. E., U. S. Dept. Agr., *Bull.* 89 (Nov., 1920; revised Sept., 1921).

Rapid Method for Calibration of Flowmeters

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IT OFTEN becomes necessary to construct and calibrate a flowmeter or to calibrate a capillary tube for a flowmeter with interchangeable capillaries in a laboratory where no calibration apparatus is immediately available.

Under such circumstances the most useful method of calibration for laboratory meters using relatively low pressures consists of displacement of the gas by a liquid, thus forcing the gas through the flowmeter. The following advantages are obtained: simple construction from ordinary laboratory apparatus; precision, accuracy, and rapidity of calibration measurements due to upward displacement of gas; and economy of calibrating liquid (if other than water is used).

Most methods (*1*) displace a liquid, such as water, by the gas after the gas has passed through the flowmeter. If at the end of a known time the volume of liquid used is measured, the volume of gas flowing per unit time can be calculated. However, in such techniques downward displacement of liquid tends to create a back pressure which changes the rate of flow as the head of the liquid changes.

As it was necessary to carry out a number of calibrations, the following procedure was devised:

An arrangement *B* and *C* (Figure 1), giving a constant head (or any such device), allows water to run into a calibrated mixing cylinder of 1- or 2-liter capacity. *B* may be an inverted 2-liter bottle with the bottom removed. The stream of water is controlled by means of a stopcock or screw clamp, *A;* a stopcock is more satisfactory. The water displaces the sample of gas in the cylinder, forcing it through the flowmeter. Since the inlet tube into the cylinder is bent, there is no splash, and readings are easily made on the cylinder. When *A* is adjusted to a convenient pressure differential, indicated by the liquid in the flowmeter, a stop watch is started and the level in the cylinder is noted. This is usually about 200 to 300 ml., so that a large cylinder calibrated like a graduate of the same size is desirable.

From these measurements the volume per unit time flowing through the flowmeter can be calculated. This operation is repeated for various pressure differentials of the indicating liquid in the flowmeter by regulating *A*. It is convenient to construct a graph, plotting pressure differential against volume per unit time.

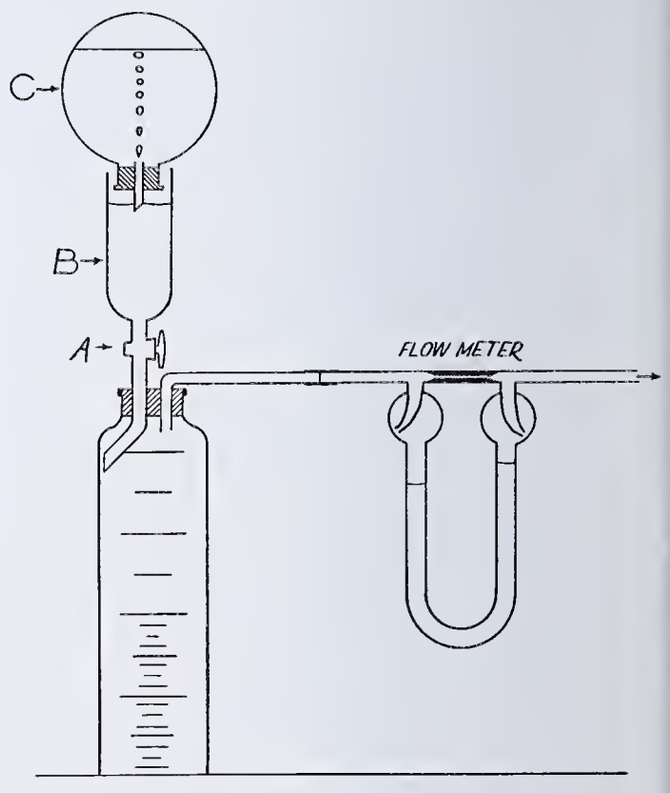

FIGURE 1

The design of the apparatus permits, with slight modification, use of any gas desired.

If water is undesirable as a calibration liquid, mercury or oil may be substituted, the apparatus being designed so that there is no loss of liquid.

The dropping mercury method, on a smaller scale than herein described, is especially adapted to the calibration of sensitive meters, measuring the continuous flow of small quantities.

Literature Cited

(1) Smith, G. W., IND. ENG. CHEM., Anal. Ed., 4, 244 (1932).

[1] Present address, U. S. Food and Drug Administration, Federal Office Bldg., San Francisco, Calif.

Carbon Train for Control Analysis

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OF THE methods now being used for the determination of carbon in steel, the combustion method has proved most satisfactory from the point of view of accuracy and general applicability. On occasion, however, as for some purposes of control work, greater speed is demanded than that afforded by the ordinary combustion method. It is the purpose of this paper to show how, by an adaptation of the ordinary combustion train, more rapid analyses may be obtained.

Practically all texts of quantitative analysis include discussion of the ordinary combustion method for carbon in steel and a description of a carbon train. This being the case, only those modifications of the train will be discussed which serve to speed up the analysis.

Modifications of Train

HASTENING THE SWEEPING PROCESS. A large part of the time taken for an analysis is used in the sweeping process, in which the gaseous products formed in the combustion of the sample must be swept out of the combustion tube and absorbed. This sweeping period may be considerably shortened by reducing the volume through which the gas must be passed. Such volume reduction is effected by inserting in the exit end of the tube a quartz plug (*A*, Figure 1), made by sealing off both ends of a piece of quartz tubing. The outside diameter of the plug should be only slightly less than the inside diameter of the combustion tube. In the train described, the quartz plug was 2.34 cm. (0.906 inch) in outside diameter and the inside of the combustion tube was 2.5 cm. (1 inch).

REMOVAL OF SULFUR GASES AND WATER VAPOR. Following the combustion tube is an absorption tube, *B*, of about 50- to 60-cc. capacity which is attached to the combustion tube by means of a short piece of heavy-walled rubber tubing. It is half filled with 20-mesh zinc to remove sulfur gases (*1*) and the balance is packed with Anhydrone.

ARRANGEMENT OF TRAIN. Considerable time may be saved by setting up the carbon train for easy manipulation. Following the three-way stopcock (Figure 2) is a 0.47-cm. (0.188-inch) copper tube, *E*, rigidly secured by means of a table clamp, *F*, rod, *G*, and clamp holder, *H*, and extended into and close to the base of the balance. With this arrangement it is necessary to hold the balance door only slightly open during weighing. This narrow opening does not sensibly affect the weighing.

The copper tube in the balance terminates in a one-hole rubber stopper, *C*. The tube extends half way into the stopper, leaving the other half to accommodate the weighed absorber, *B*. The combination of copper tube and stopper should be rigid enough to act as the only support of the weighed absorber. Using this support, the absorber can be handled with one hand.

A sketch of the weighed absorber, including dimensions, is seen in Figure 3. Another absorber of the same dimensions, also filled with Ascarite and Anhydrone, is used as a counterpoise (*A*, Figure 2). This counterpoise is necessary, as the effluent gas, oxygen, exerts an increased buoyant effect on the weighed absorber (*B*, Figure 2) over that of air. The absorbers may be interchanged when the weighed absorber is exhausted. Both are left unstoppered during the entire time of an analysis. Weighing with the absorber open introduces no error.

METHOD OF WEIGHING. Since stress is placed upon speed, it

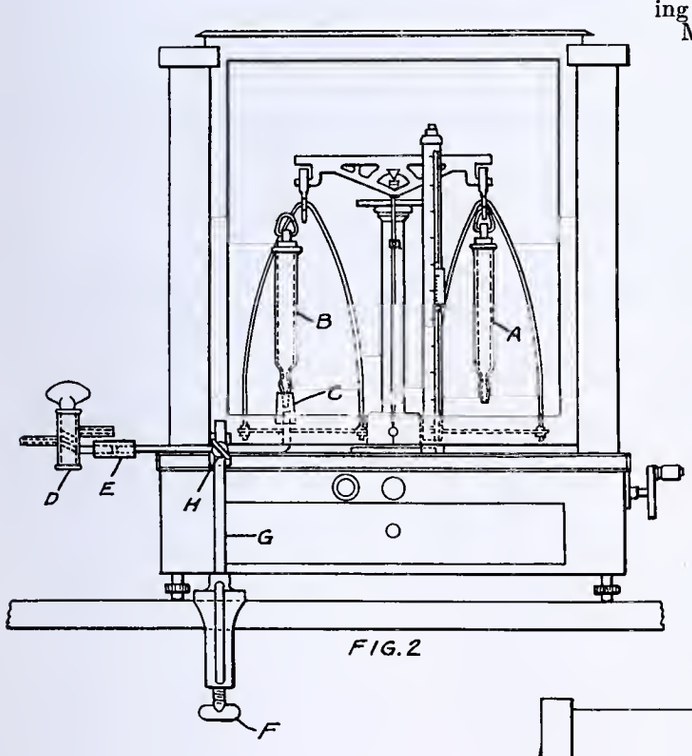


FIG. 2

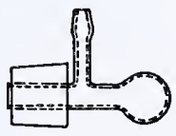


FIG. 4

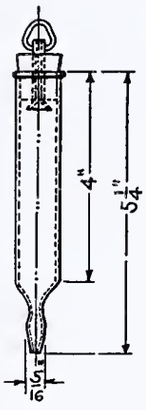


FIG. 3

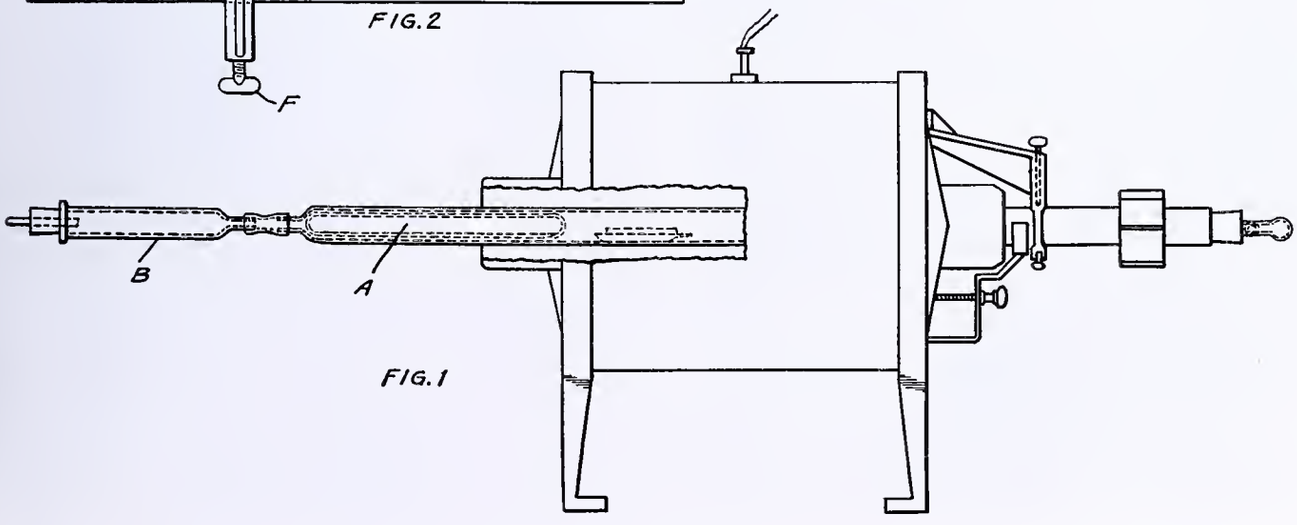
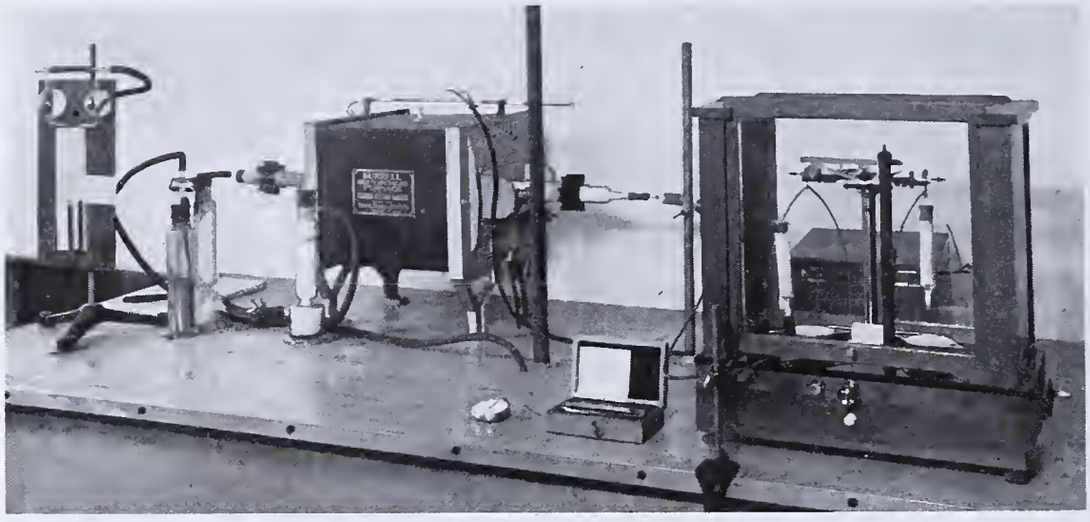


FIG. 1



Set-up of Carbon Train

is necessary to use a fast method of weighing; accordingly, the single deflection method (*2*) was employed. This method is rapid and does not involve a loss in sensitivity as when using a magnetic damper. The authors used a chainomatic balance of ordinary sensitivity (0.25 division per 0.1 mg).

Boats and Covers. For combustion of the sample an Alundum boat 8.75 cm. (3.5 inches) long, 1.25 cm. (0.5 inch) wide, and 0.78 cm. (0.31 inch) deep is used. The boat is provided with an Alundum cover to protect the combustion tube from flying particles of ignited oxide. By eliminating the bedding material, a more rapid ignition of the sample is obtained, but it is necessary to use a new boat every third or fourth analysis. Prior to use, a large number of boats and covers are burned out at 900° to 1000° C. for about 0.5 hour in a muffle furnace, and are then stored in a large desiccator until ready for use.

Loading the Boat for Analysis. In the boat is placed a 0.500-gram steel sample together with approximately 0.5 gram of tin shot, which promotes rapid combustion of the sample. The combustion tube is kept at a temperature of 1150° to 1200° C., using a furnace with globar heating elements.

Purification and Metering of Gas. The following devices are used for the regulation and purification of oxygen: a gas regulator for the oxygen tank (an Air Reduction Company combination gage and regulator was found very satisfactory); an ordinary calibrated U-tube gage to measure the gas flow; a soda-lime tower to remove carbon dioxide; and a tower of Anhydrone to remove moisture.

It is also advisable to provide the combustion tube with a clear glass window. A simple and inexpensive device is the glass T-tube of Figure 4, which fits into the rubber stopper of the combustion tube. A bulb is blown on the glass T-tube, so that the operator may see the boat in the combustion tube.

Procedure for Analysis

The gas flow from the oxygen cylinder is adjusted so that it is between 250 and 300 cc. per minute. No further adjustment of gas flow is made, except that necessary to maintain the specified flow rate. At the beginning of a series of determinations the weighed absorber is set in place on the train in the rubber stopper (*C*, Figure 2) and flushed with oxygen for several minutes. This preliminary flushing is necessary only when the absorption tube has been allowed to stand in the air for some time, so that appreciable diffusion has taken place. When the absorber is to be weighed the three-way stopcock (*D*, Figure 2) is turned so that oxygen from the combustion tube exhausts into the air; weighing while a stream of gas is directed into the balance would not be advisable. The absorber is now taken from the stopper, suspended from the stirrup of the balance, and weighed. It is then replaced on the train and gas is passed through it by proper adjustment of the stopcock.

One-half gram of tin is placed in the boat, the cover is put in place, and the boat and contents are pushed into the combustion tube. The boat is pushed as close as possible to the quartz plug without at the same time moving it out of the hot zone of the combustion tube (Figure 1), in order to make rapid flushing possible. With the temperature of the combustion tube between 1150° and 1200° C. in every case the sample burned rapidly. Rapid burning is attended by a stream of glowing oxides from the sample.

The time that elapses after introduction of the boat into the combustion tube, before rapid ignition sets in, varies somewhat with the composition of the steel, the size of the sample, and its form (shot, drillings, etc.). In the case of drillings, for plain carbon steels, rapid ignition sets in within 30 seconds after introduction of the sample. Using the Bureau of Standards samples, which are millings, between 30 and 60 seconds elapse before rapid combustion sets in. In the case of shot, which is the form in which the control chemist usually receives the sample for analysis, a sample of 12- to 14-mesh is suitable. Usually one minute is necessary to burn the sample completely after ignition once starts. At the end of this time, the tube must be flushed for 30 seconds. After flushing, the oxygen is exhausted into the air and the absorber is immediately weighed.

Thus the absorber is ready for weighing 2.5 minutes after introduction of the sample into the combustion tube. The total time of an analysis will depend on the skill of the operator. However, using the carbon train described and weighing by single deflection, an analysis can be made in 4 minutes.

In the manipulation of the train, several features represent a departure from conventional practice. No attempt is made to regulate the gas flow during the analysis. The flow rate is set at the start of a series of analyses and the only change is the path the gas takes, as controlled by the three-way stopcock (*D*, Figure 2). During a combustion, the gas is passed through the absorber; while the absorber is being weighed the gas is allowed to escape into the air. The absorber is at all times kept in the balance case, which minimizes any error in weighing due to temperature difference between absorber and balance. The absorber is left unstoppered at all times and weighings are made as rapidly as possible.

Discussion of Results

Table I contains the results obtained using Bureau of Standards samples. These include two alloy steels which are difficult to burn and a cast iron. With the exception of

Table I. Determination of Carbon

Sample No.	Bureau of Standards Certified Value	Carbon Present %	Carbon Found %	Error %
		Plain Carbon Steels		
11d	0.202	0.20	0.21	+0.01
	0.202	0.20	0.20	None
	0.202	0.20	0.21	+0.01
12d	0.418	0.42	0.40	−0.02
	0.418	0.42	0.41	−0.01
100	0.617	0.62	0.63	+0.01
	0.617	0.62	0.62	None
130	0.454	0.45	0.45	None
	0.454	0.45	0.46	+0.01
72a	0.317	0.32	0.32	None
14b	0.817	0.82	0.81	−0.01
	0.817	0.82	0.80	−0.02
72	0.294	0.29	0.30	+0.01
	0.294	0.29	0.30	+0.01
15b	0.101	0.10	0.10	None
	0.101	0.10	0.10	None
13c	0.573	0.57	0.55	−0.02
	0.573	0.57	0.57	None
		Alloy Steels		
121 (CR 18%, Ni 8%)	0.057	0.06	0.07	+0.01
	0.057	0.06	0.07	+0.01
126 (Ni 36.4%)	0.034	0.03	0.03	None
	0.034	0.03	0.04	+0.01
		Cast Iron		
5g	2.86	2.86	2.91	+0.05
	2.86	2.86	2.88	+0.02
				Av. ±0.01

the cast iron, which showed in one determination an error of +0.05 per cent, the greatest error for any determination was 0.02 per cent. Most of the analyses show either an error of 0.01 per cent or no error at all.

The certificate values of the second column are rounded and listed as percentage of carbon present in column 3. It is these latter values that are compared with the results obtained, because the magnitude of the weight change in the absorber restricts expression of results to two significant figures.

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(2) Brinton, P. H. M. P., *J. Am. Chem. Soc.*, 41, 1151 (1919).

Opinions expressed in this article are those of the writers and do not necessarily reflect the ideas of the Naval Service.

Simple Tests to Indicate the Condition of an Analytical Balance

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IT OFTEN falls to the lot of a teacher of quantitative analysis or an industrial chemist to determine whether a balance is in satisfactory weighing condition. This paper presents a simple and rapid method of balance testing that is sufficiently exacting for most purposes. The fundamental principles upon which this brief series of tests is based are well recognized, but the method of application is believed to be new. A recent paper (*1*) gives valuable additional information from the instrument repairman's point of view.

The following points should be considered:

1. The general condition of the balance must be acceptable.
2. The rest point should be constant for any particular load when the mass on each of the pans is equal.
3. The balance must be of the proper degree of sensitivity. (Sensitivity is understood in this paper to be numerically equal to the deflection on the pointer scale caused by the addition of a 1-mg. load to a single pan of the balance.)
4. The balance must give weighings that are closely reproducible.
5. The balance must have lever arms of nearly equal length.

These points are intimately interrelated—for example, an imperfect knife-edge may cause the balance to perform poorly in respect to points 2, 3, and 4.

Point 1 is not readily tested quantitatively, but a careful inspection will usually suffice.

Make sure that the beam releases, pan rests, rider carrier, chain weight devices, and other moving parts are in good mechanical condition. See that the knife-edges are separated from their bearing plates by the beam lift to a gap of about 0.1 mm. and that when the beam lift is released all three knife-edges make contact with their plates over the whole edge gently and simultaneously. This is essential to the life of the edges. Metal parts well finished and free from corrosion are desirable, but do not necessarily indicate an accurate balance.

The information sought in points 2 and 3 can be obtained quantitatively by the following method:

Place the balance on a firm support in a part of a room where a fairly constant temperature prevails (away from radiators, open windows, and other drafts, out of direct sunlight, and removed from other hot light sources). Level the balance with the setscrews provided in the base. If the balance has recently been moved from another location, open the door of the case and allow at least an hour for the balance to attain room temperature.

After the balance has been brought to the same temperature as its environment, determine the data required to construct a table similar to Table I. For this purpose select two sets of analytical weights, W_1 and W_2. It is convenient, but not necessary, to have the sets agree within fairly narrow limits. They need not be calibrated. If two sets of weights are not available, one can make shift with only one set—for example, if the data at 20 grams' load are to be determined, one could call the two 10-gram weights together W_1 and the 20-gram weight W_2.

Table I records data obtained in applying this method to a typical student balance. The principle involved is that of double weighings first devised by Gauss.

Table I. Data for Student Balance

Weight of W_1 and W_2 (Each) *Grams*	Rest Point[a], *A*	Rest Point[b], *B*	Average Rest Point, *C*, $(A + B)/2$	Rest Point[c], *D*	Sensitivity, *E* $(B - D)$
0	9.0	9.0	9.0	6.9	2.1
10	9.5	9.1	9.3	7.3	1.8
20	10.2	8.6	9.4	7.2	1.4
50	9.7	9.5	9.6	8.5	1.0
100	9.3	11.1	10.2	10.3	0.8

[a] W_1 on left pan, W_2 on right pan.
[b] W_2 on left pan, W_1 on right pan.
[c] W_2 on left pan, W_1 + 1 mg. on right pan.

Constancy of the values in column *C* would satisfy point 2. If the rest point in *C* should shift by as much as two or three pointer scale divisions between loads of zero weight and 100 grams' weight on each pan, the balance would not be acceptable for determining absolute mass values, but might prove acceptable for certain types of gravimetric analysis where the determination of small differences in mass only is required. The balance tested (Table I), where the rest point shifts 1.2 scale divisions between zero weight and 100 grams' weight load on each pan on the basis of a sensitivity of 0.8 at 100 grams' load, would cause an error of 1.5 mg. in determining a 100-gram load. This amounts to a deviation of only 0.0015 per cent, which would be negligible for most work.

It is worth while to test the effect of changing the position of the masses from the centers of the pans to the edges and see if the value of the rest point is thereby changed. Defects of the end knife-edges may sometimes be detected by this method, whereas they may remain unnoticed when the masses on the pans are perfectly centered. The point of rest should also be checked by using swings of small amplitude and then swings of considerably greater amplitude. Difference between the two values indicates worn, nonparallel, or otherwise faulty knife-edges.

If the balance is to be used where the requirements are only moderately exacting (point 3), the sensitivity (column *E*) should have a numerical value of at least 2 and preferably 3 or 4 at zero load on the balance pans. The sensitivity of a balance should remain nearly constant or should decrease slowly and regularly with increasing load on the balance pans. The fall in sensitivity is usually due to a difference in level between the middle and the end knife-edges, and may be caused by bending of the beam under the load, by wear of the knife-edges, or by not sharpening them uniformly. In general, it is not safe to use a balance for loads that reduce the sensitivity to less than 40 per cent of the value with zero load.

Weights of 90 to 100 grams should be the maximum allowed on each pan of the balance that is serving as our example.

Point 4 could be determined by again getting the rest point of the balance with pans empty after securing the data of Table I. If this checks with the corresponding value in the table within 0.2 pointer scale division, the balance is satisfactory. If further checks on this point are desired, one can repeat the weighings at any pan load.

Point 5 is easily tested as follows:

An object of mass M (a 20-gram weight is convenient) is placed on the left-hand balance pan and counterbalanced with other weights from the set and their sum, S, is recorded. M is then transferred to the right-hand balance pan and again counterbalanced with weights from the set and their sum, S', is recorded. If L equals the length of the left lever arm and R equals the length of the right lever arm of the balance, from the principle of the lever

$$ML = RS \quad (1)$$

and

$$MR = LS' \quad (2)$$

If we divide Equation 2 by Equation 1 we get

$$R/L = \sqrt{S'/S} \quad (3)$$

On developing the quantity under the radical sign in terms of a series of powers of x and S, where x represents the difference between S' and S ($S' = S \pm x$), we get the series

$$\frac{R}{L} = 1 \pm \frac{x}{2S} \mp \frac{x^2}{8S^2} \pm \frac{x^3}{16S^3} \cdots\cdots \quad (4)$$

In applying this to the balance, x is always very small as compared to S; so the equation reduces essentially to

$$\frac{R}{L} = 1 \pm \frac{x}{2S} \quad (5)$$

The upper sign is used where S' is greater than S and the lower sign is used when S' is less than S. In a good balance the R/L value should be 1.0 ± 0.00002. In dealing with comparative values, as in gravimetric analysis, an R/L value of 1.0 ± 0.0002 can be tolerated without appreciable error in the final result.

In deciding whether a balance is suitable for the work at hand, one must also know the probable limits of error introduced by factors other than the balance. The balance may be used without hesitation if it is twice as accurate as the least accurate of any of the other measurements involved. It is very probable that more errors in student and commercial work are due to uncalibrated or poorly calibrated weights than to inaccurate balances. Moreover, manipulative techniques, aside from weighing, usually introduce far larger errors than can be accounted for by the inaccuracy of weighing; and the percentage of error of many analytical methods, due to such things as end-point errors, solubility of precipitates, adsorption, deliquescence, inability to measure volumes accurately, etc., is far greater than most of us would tolerate in an analytical balance.

Literature Cited

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Useful Centrifuge Accessories

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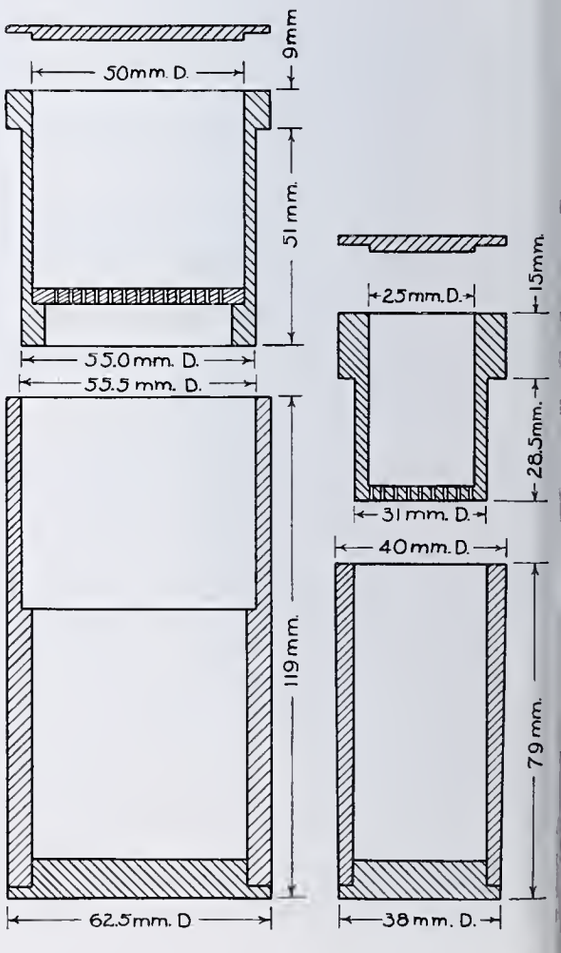
WHEN reagents are to be purified rapidly and efficiently by crystallization, centrifugal draining of the crystals is essential. Unfortunately, convenient and inexpensive centrifuge accessories for this purpose are scarce, particularly for crystallizations which must be carried out on a small laboratory scale. The basket-head type of apparatus is expensive, cannot easily be made without special equipment, and is not very convenient for use with small quantities of material, or in any case where very high purity is required. Accessories which permit the adaptation of various sizes of Büchner funnels or Gooch crucibles as baskets are not quite so expensive, but are unsatisfactory in other respects.

The perforated cup type of accessory, in which both crystals and liquid are completely enclosed during centrifuging, is especially suitable for small quantities of material. Made from platinum or gold, such an accessory is universally useful, but the cost is prohibitive for most laboratories. A plated apparatus might seem to offer a suitable substitute, but inquiries indicate that the cost of any practical design is rather high. Accessories of this type made from the methyl methacrylate resin Lucite are inexpensive and for many purposes fully as useful as if they were made from one of the noble metals. Lucite is almost completely insoluble in water solutions of salts, acids, and dilute alkalies, and in straight-chain hydrocarbons. However, it cannot be heated much above 70° C. and is soluble in many organic solvents.

The authors have designed two accessories which may be machined from stock sizes of Lucite sheet, rod, and tubing to fit standard centrifuge cups. The inner cup of the small model has 61 holes drilled in a hexagonal pattern with a No. 70 B. & S. gage drill. The inner cup of the larger model has interchangeable bottom plates, each with 169 No. 60 or No. 70 holes.

Eight complete accessories were made for $3.50 each, including the cost of labor and material. This is one eighth the lowest quotation obtained for a single set made from any suitable combination of base metals, and one fifteenth the cost of a small basket-head accessory made from manganese bronze. The economy is even greater, since the Lucite accessories are more generally useful with water solutions than those made from base metal alloys.



Ultraviolet Photometer

Quantitative Measurement of Small Traces of Solvent Vapors in Air

V. F. HANSON, E. I. du Pont de Nemours & Co., Inc., Niagara Falls, N. Y.

An ultraviolet photometer has been developed for rapidly measuring concentrations of trichloroethylene as low as 10 p. p. m. and perchloroethylene as low as 0.3 p. p. m. in air. It is applicable to many toxic organic vapors in concentrations as low as 0.13 p. p. m. Its operation depends on the partial opacity of these vapors to ultraviolet light. It is specific for vapors having a strong absorption in the 2537 Å. region of the spectrum (Table I). Special measures must be taken when mixtures of such substances are present.

The instrument is portable, direct-reading, and capable of making at least two determinations per minute on substances for which it has been previously standardized.

Details of principles of operation, construction, and application are given.

THE increasing use of chlorinated solvents, particularly trichloroethylene and perchloroethylene, in degreasing, dry cleaning, and extraction has led to an urgent need for a rapid and reliable analytical method for determining solvent concentrations in the air in the working areas.

Such an analytical method is needed in studying the causes of solvent losses for both economic and health hazard reasons. Results of analyses should be instantly available to check changes in concentrations during the various operations. The method should be simple, so that special technicians are not required, and the equipment should be portable and sensitive to concentrations as low as 20 parts per million. Health, insurance, and labor agencies of several states have requested some of the operators using these solvents to check their equipment to preclude the possibility of health hazards arising as a result of excessive solvent losses, which are generally due to faulty operation or defective equipment.

Methods of Analyses Extant

The following analytical methods have been used with varying degrees of success:

1. Thermal decomposition of the solvent followed by absorption and measurement of the products of decomposition (*4, 8, 9, 12*)
2. Alcoholic absorption-colorimetric method (*1*)
3. Specific gravity measurements (*5*)
4. Refractive index measurement (*6, 10*)
5. Weight gained by charcoal absorption (*2*)
6. Thermal conductivity (*14*)
7. Vapor pressure of condensed solvent (*3, 7, 11*)
8. Flame tests (*13*)

A critical inspection of these methods indicated that none fully met the requirements set forth above. Therefore, the R. & H. Tri-Per-Analyzer was developed to determine microquantities of trichloroethylene and perchloroethylene (known as "Tri" and "Per" to the trade) in air.

It consists of an extremely sensitive and stable ultraviolet photometer with a built-in sampling and standardizing system. Its operation depends on the partial opacity of solvent vapors to certain bands of light in the ultraviolet region of the spectrum. Ease of operation makes it possible for a layman to make a complete survey of solvent concentrations in an area of a plant in a half hour's time. Concentrations from 10 to 2000 p. p. m. of trichloroethylene and 0.3 to 500 p. p. m. of perchloroethylene can be measured, and higher concentrations can be determined by making slight modifications in the instrument. It is common practice to make two determinations per minute of trichloroethylene vapor in concentration as low as 10 p. p. m. (by volume) or as low as 0.3 p. p. m. of perchloroethylene vapor in air. The analyses are made as rapidly as samples can be pumped through the instrument, the results of the analyses being instantly available.

TABLE I. SENSITIVITY OF PHOTOMETER

	Sensitivity, per Scale Division *P. p. m.*
Mercury	0.0001 (approx.)
Tetraethyllead	0.13
Xylene	0.2
Monochlorobenzene	0.3
Aniline	0.3
Perchloroethylene	0.5
Chloroprene	0.5
Toluene	1.0
Benzene	1.2
Vinylacetylene	1.6
Phosgene	5
Acetone	5
Ethylbenzene	5
Pentachloroethane	7
Hydrogen sulfide	8
Trichloroethylene	10
Carbon disulfide	12
Gasoline (Blue Sunoco)	50

Photometer Insensitive to:

Methylene chloride	Ethyl alcohol
Carbon tetrachloride	Amyl alcohol
Ethylene dichloride	Ethyl acetate
Tetrachloroethane	Ethyl Cellosolve
Chloroform	Methyl Cellosolve
Methyl chloride	Dowtherm A
Vinyl chloride	Water vapor
Methyl alcohol	

While the instrument was developed primarily for trichloroethylene and perchloroethylene, it may be used equally well on many toxic vapors, but is insensitive to others (Table I).

An instrument operating on similar principles has been used for measuring mercury concentrations in air (*15*).

Theory of Operation

All substances absorb light at some region of the spectrum. If a spectral region exists where a vapor has a high absorption and the diluent a high transmission, the concentration of the vapor can be measured in terms of light absorbed. The ideal instrument would be one using a monochromatic source of light of wave length corresponding to the maximum absorption of the vapor and a minimum absorption of the diluent and a highly sensitive and stable light-measuring device sensitive only to this wave length.

The operation of this analyzer is based on the Beer-Lambert law which states that the light absorption depends on the distance traversed and the molar concentration of the light absorbent. Thus, a unit layer of unit concentration absorbs as much light as a layer of twice the thickness and half the concentration. The Beer-Lambert law is then

$$I = I_0 10^{-dca}$$

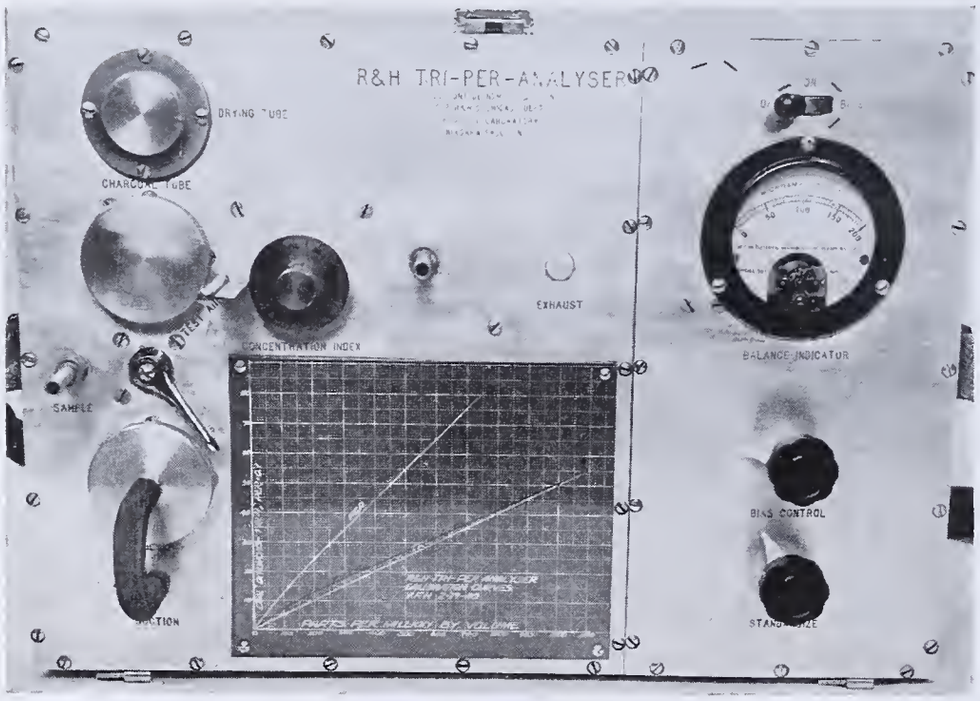


FIGURE 1. PHOTOMETER

where I is the intensity of the transmitted light, I_0 is the intensity of the incident light, a is the extinction coefficient for common logarithms, c is the molar concentration of the light absorbent, and d is the thickness of the layer measured in centimeters.

It is very important to note that these relations favor measurements of low concentrations of the light absorbent—i. e., there is a greater percentage difference in light absorbed by small quantities than by larger quantities of absorbent.

The other analytical methods used for measuring small traces of vapors operate on a linear relationship, and have the obvious disadvantage for low concentrations of giving the same incremental differences regardless of concentration. Thus, while a unit absolute error in a reading at high concentrations might result in a low relative error, the same absolute error at low concentrations would result in a high relative error.

The following average extinction coefficients were found to hold for a light consisting of 90 per cent 2537 Å. radiation (extinction coefficients, a, are calculated for log base 10): trichloroethylene, 1.9; perchloroethylene, 45.

Selecting the following requirements:
Light cell length, d, 15 cm.
Minimum concentration of trichloroethylene to be detected, 10 p. p. m.
Minimum molar concentration

$$\frac{10^{-5}}{22.4} = 4.45 \times 10^{-7} \text{ mole per liter}$$

then $$I = I_0 10^{-acd} \quad (3)$$

$$\text{Log } 10 \frac{I_0}{I} = acd$$

so that

$$\text{Log } 10 \frac{I_0}{I} = 1.9 \times 4.45 \times 10^{-7} \times 15 = 1.27 \times 10^{-5}$$

the ratio of light transmitted by solvent-free air to air containing 10 p. p. m. of trichloroethylene, is approximately $\frac{I_0}{I} = 1.00003$.

It is thus evident that the photometer must be capable of detecting changes in light intensity of 0.003 per cent in order to detect 10 p. p. m. of trichloroethylene.

The change in transmitted light due to the presence of solvent vapors is measured by the change in resistance of a sodium photocell whose peak sensitivity is at about 3000 Å. Instead of measuring the voltage drop across a fixed resistor in series with this photocell, a second photocell is used as both a series resistor and as a means for partially compensating for changes in the light output from the low-pressure mercury vapor lamp. The photocell voltage is impressed on the grid of a battery-operated pentode which amplifies the changes in photocell voltage. These changes are indicated on a microammeter in the plate circuit of the tube. All measurements are made when the light to the two photocells is equal, to eliminate errors due to changes in battery voltage and tube characteristics.

R. & H. Tri-Per-Analyzer

The instrument and accessories are self-contained in a carrying case weighing 16 kg. (35 pounds). Figure 1 shows the instrument set up for use. Figure 2 shows the panels removed from the case.

ANALYZER UNIT. The analyzer unit is composed of the following elements:

Optical and Light-Measuring Systems. Low-pressure 5-watt mercury vapor lamp emitting 2537 Å. ultraviolet light, lamp stabilizer networks, 15-cm. sample cell provided with quartz windows, two General Electric FJ76 sodium phototubes operating in a bridge network, vacuum tube electrometer with a microammeter balance indicator, and micrometer "light valve".

Sampling System. Charcoal absorption tube, porous filter, sampling pump, and control valve.

The schematic drawing (Figure 3) shows the arrangement of the equipment.

ELECTROMETER BALANCE INDICATOR. An RCA32 tube operating at reduced heater and plate voltage to reduce grid current and increase stability is mounted near the phototubes. The tube control leads pass through the partition into the battery compartment. Grid bias control and phototube balancing potentiom-

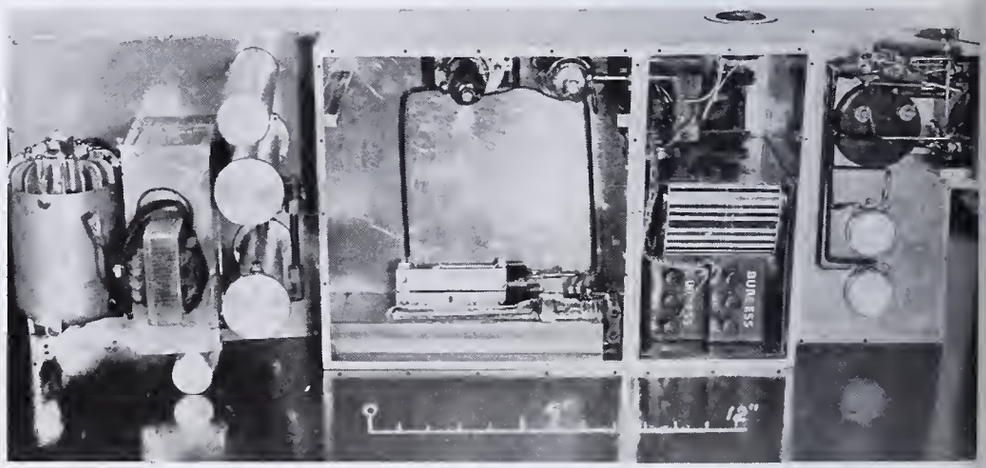


FIGURE 2. INSTRUMENT WITH PANELS REMOVED FOR INSPECTION

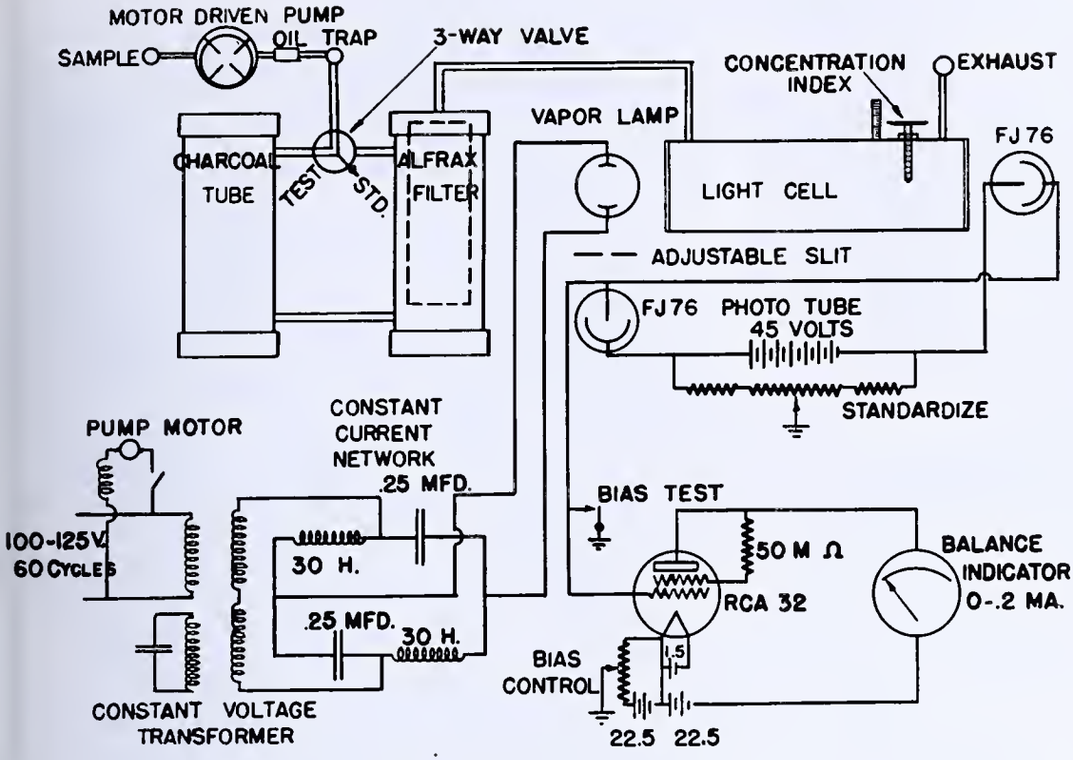


FIGURE 3. SCHEMATIC DIAGRAM OF PHOTOMETER

ters with the plate microammeter are mounted in the battery ompartment. A switch is provided to ground the grid to tandardize the electrometer.

The combination of voltage and current control with the com-ensating reference photocell, integral sample cell, and light valve nd rapid sampling and standardizing systems were all found ecessary to provide the sensitivity and stability to measure hanges in light intensity of less than 0.003 per cent.

OPTICAL AND LIGHT-MEASURING SYSTEMS. After attempting o use light-splitting, collimating, and optical wedges for in-reasing and distributing the light to the two photocells, the imple optical system shown in Figures 2, 3, and 4 was adopted. While greater sensitivity could be obtained with a longer light ell than the 15 cm. used, the decrease in light intensity on the hotocells reduced the instrument stability, more than offsetting he advantage of increased sensitivity. Space was also consid-red an important factor in designing a portable rugged field nstrument.

The light from the 5-watt General Electric sterilization lamp asses through the light cell (Figure 4) which is mounted on the nstrument panel. Light is also transmitted to the reference hototube through an adjustable slit below the lamp (Figure 2). The light to the two phototubes is approximately equalized by djusting the light slit on the reference photocell. The fine alance is attained by the voltage divider ("Standardize", Figure) across the phototube battery. This fine balance consists of a adio potentiometer in series with two fixed resistors to limit the ange of the electrical adjustment. The light is thus balanced vhen the voltage drop from the common wire of the phototubes o the ground is zero.

The simple micrometer screw proved to be a very satisfactory nethod for measuring the light that was absorbed.

In operating the instrument, the two phototubes are first alanced as described, with solvent-free air in the test chamber nd the "Concentration Index" set at zero. Then the instru-nent three-way valve is switched to "Test Air", and the "Con-entration Index" micrometer is raised until the original balance s restored. Thus, by decreasing the area of the shadow cast by he "Concentration Index" screw on the phototube, the amount f light that is absorbed by the test air is compensated for in a nanner that permits very simple measurement.

One complete turn of the "Concentration Index" dial elevates he screw 1 mm. The dial, being divided in 100 parts, makes a hange of 0.0031 per cent per dial division on the photocell based n the ratio of the area of the shadow cast by the screw on the hototube to the area of the phototube element.

Concentrations of various vapors in terms of "Concentration ndex" dial divisions are plotted on Figure 5. For convenience, alibration curves for the trichloroethylene and perchloroethylene re mounted on the instrument panel.

Since it is impossible to secure equally balanced phototubes, special care was required to stabilize the ultraviolet lamp, which was found to be extremely sensitive to voltage and temperature changes. The lamp has the negative volt-ampere characteristic of an electric arc, making it necessary to provide a constant current network to maintain a steady current regardless of changes in lamp voltage and impedance arising from changes in lamp pressure and temperature. A resonant circuit consisting of equal inductive and capacitive reactances connected in the form of a square was found to give excellent current control as long as the line voltage was constant. This network was made up from radio chokes and by-pass condensers, the impedance of each component being equal to the lamp impedance. These elements were selected to operate the lamp at about half the normal current to minimize changes in lamp characteristics with time.

A Sola constant-voltage transformer proved satisfactory for controlling the lamp voltage in spite of normal variations in line voltage.

SAMPLING SYSTEM. The successful use of the analyzer depended as much on the sampling system adopted as on the optical system.

The sample is pumped first through a charcoal tube which removes all traces of solvent vapors. A tube 3.12 cm. (1.25 inches) in inside diameter and 15 cm. (6 inches) long was found effective in reducing solvent concentrations from 600 to less than 1 p. p. m. After the instrument is balanced at the zero point, the three-way valve is turned so that the sample by-passes the charcoal tube, passing through a porous Alfrax filter before it enters the sample cell. This filter is mounted on the screw top of the filter tube, from which it is readily removable for cleaning and inspection. The frequency of cleaning depends on the type of atmosphere to which the instrument is subjected. Unless paint spray, oil, or similar contaminant is encountered, it has been necessary to clean the cell about three times per year.

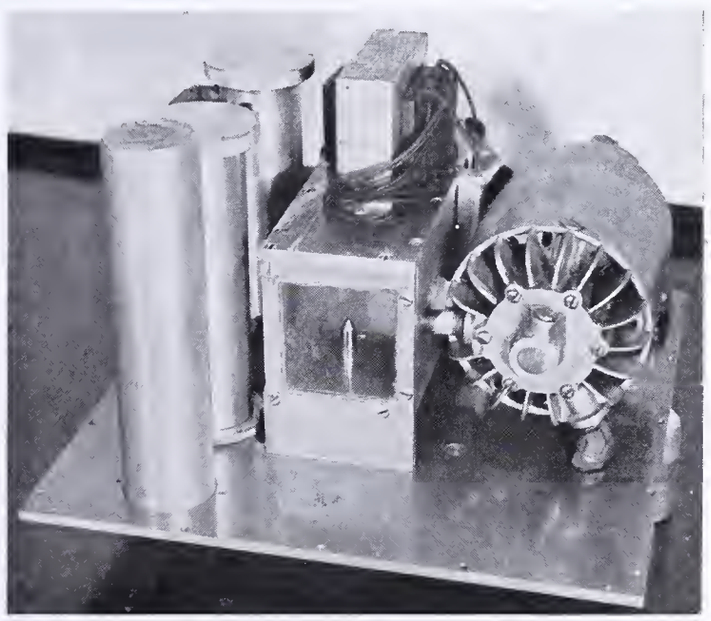


FIGURE 4. DETAILS OF SAMPLING SYSTEM

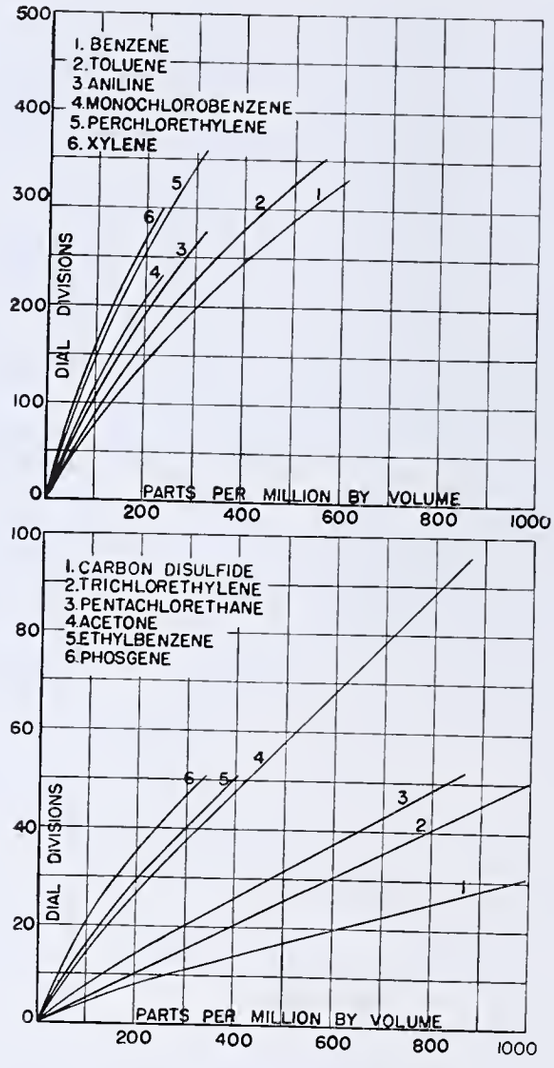


FIGURE 5. CALIBRATION CURVES

Accurate results depend on rapid circulation of the sample through the instrument. Changing the sample about 20 times per minute permits frequent checks of the zero point, which is important for attaining high accuracy at low concentrations giving up to 10 scale division deflection. Motor-driven pumps were troublesome because of lubrication; however, a small cotton filter was effective in keeping the oil from being blown by the pump into the instrument. It was found necessary to force the sample through the cell in order to maintain substantially atmospheric pressure on the sample. Suction of the sample through the cell was unsatisfactory because of cell pressure variations.

Calibration and Accuracy of Analyzer

The analyzer is periodically checked by adding carefully measured quantities of liquid solvent to a 850-liter (30-cubic foot) fumatorium in which an electric circulating fan is mounted. After the solvent sample has been thoroughly mixed with the air, it is passed through the instrument. At least three readings are made at each concentration, and the average dial readings are then plotted against the known concentrations. Figure 5 shows the calibration curves for the materials listed in Table I, at 22° C. and 760-mm. pressure. Unless pressure and temperatures differ greatly from these values, corrections need not be applied. The instrument is about 25 times as sensitive to perchloroethylene as to trichloroethylene.

The instrument has been checked six times in the year that it has been in service, during which it has traveled over 50,000 miles without serious accidents that affected its accuracy. Except for one occasion when a gummy film was found on the quartz windows, the recalibration checked the initial calibration within 2 scale divisions up to concentrations of 1000 p. p. m. of trichloroethylene.

There is a gradual decrease in instrument sensitivity due to changes in the spectral output of the lamp. Errors due to such changes can be minimized by making periodic recalibrations. One or two points on the curve are generally sufficient to check the calibration.

By taking the average of two or three readings, it is possible to obtain check results within one scale division on the "Concentration Index" dial. The absolute limits of accuracy vary with concentration according to the Beer-Lambert law.

For the low concentrations of vapors shown on Figure 5 the calibration curve follows a straight line from 100 to 1000 p. p. m.

Based on a tolerance of one dial division, the limits of accuracy of the instrument for trichloroethylene and perchloroethylene in air free from any other substance listed in Table I, to which the instrument is sensitive, will be:

Concentration of Vapor	Limit of Error	
	Trichloroethylene	Perchloroethylene
P. p. m.	*P. p. m.*	*P. p. m.*
0–50	±15	±0.65
50–200	±20	±0.91
200–600	±24	±0.97

The limits of accuracy of the instrument for the other substances listed would be the differences in concentrations required to produce one scale division change on the "Concentration Index".

TABLE II. FIELD TEST REPORT ON VAPOR CONCENTRATIONS NEAR DEGREASER

(Solvent used, trichloroethylene. Degreaser type, vapor-slush. Use, cleaning metals, steel. Capacity operation, 75%. Vapor concentrations, p. p. m. by volume)

Location	Machine		Exhaust System On	Exhaust System Off
			P. p. m.	*P. p. m.*
45 cm. (18 inches) above left front end	Idling		125	200
			300	170
			250	160
			220	125
		Av.	224	164
45 cm. (18 inches) above center front	Idling		190	170
			110	150
			125	150
			125	150
		Av.	128	155
45 cm. (18 inches) above right front end	Idling		170	150
			125	200
			125	220
			100	240
		Av.	128	202
Operator's nose level	Slushing		150	125
			150	300
				250
				220
		Av.	150	249
45 cm. (18 inches) above left front end	Working		360	
			200	
			380	
			310	
			340	
			2000	
			1200	
			400	
			875	
		Av.	674	

The charcoal should be changed when the zero cannot be checked when comparing clean and contaminated air. This is ordinarily about once in 20 operating hours.

Operation of Analyzer

In making a survey of concentrations in a working area near equipment using solvents to which the instrument is sensitive, as listed in Table I, the following procedure is recommended:

Allow instrument to warm up for 10 to 15 minutes. Turn on amplifier lever switch. Press lever switch to right, and set meter pointer to mid-scale (100 on a 200-microampere meter) by ad-

justing "Bias Control". Start pump, set valve on "Standard Air" and "Concentration Index" on zero. Bring meter to mid-scale by adjusting light slit for coarse adjustment and "Standardize" for fine adjustment. After meter reaches equilibrium, shift valve to "Test Air". Turn "Concentration Index" dial until meter returns to mid-scale. Return valve to "Standard Air" to check zero. Determine concentration by applying average readings to calibration curves.

The motor-driven pump should not be operated in explosive atmospheres because of sparking brushes. A hand pump is provided for such conditions.

Table II indicates the detailed data that can be obtained in less than one hour in a survey of the concentrations of trichloroethylene near a degreasing unit. The abnormally high peaks of concentration would not appear in other analytical methods listed above, except possibly with the interferometer.

Miscellaneous Applications for Analyzer

In addition to the routine analyses of air for solvent concentration, this instrument has many potential applications because of its sensitivity and speed of response. Some of these are determination of efficiency of solvent absorbents, checking efficacy of gas masks, determination of concentrations of certain vapors in gaseous products from chemical reactions, determination of concentrations of certain flammable vapors, and checking performance of fume-disposal systems.

The instrument may be modified to operate continually and may be adapted for automatic control of chemical processes or for use with other vapors.

By using selective absorbents, it is possible to determine the concentration of various components of mixtures of vapors.

Acknowledgment

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Simply Constructed Color Comparator

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THE object of a color comparator is to bring color images from reflecting or luminous surfaces into closely adjacent fields, thereby enabling the eye to compare the colors with sensitivity. These instruments are generally constructed with optical prisms. During the course of a rate study in which the progress of the process was followed by color changes, the inexpensive color comparator herein described was constructed. Its operation depends upon the fact that curved bars of certain resins will transmit light without appreciable loss through the curved surfaces. Although Plexiglas was used, any acrylate or methacrylate resin with similar optical properties would be equally satisfactory.

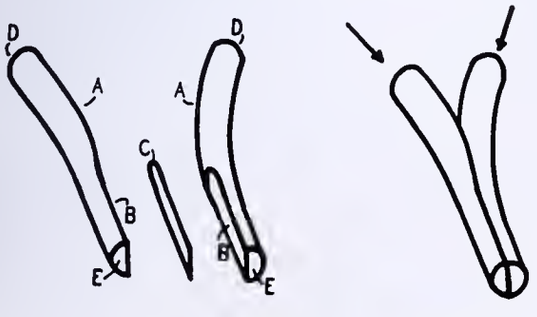


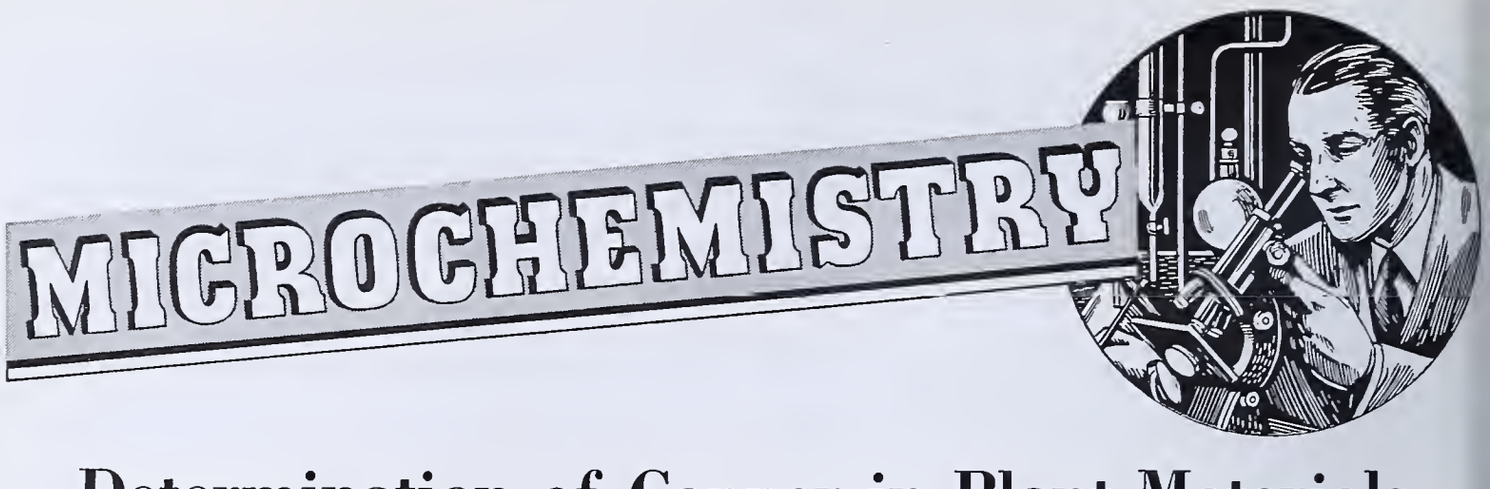
Figure 1. Parts and Assembly of Comparator

The component parts and the assembly of the comparator are shown in Figure 1. Two straight cylindrical rods of resin, *A*, 6 inches long and 0.75 inch in diameter, were softened by heating to about 110° C. and one end of each was bent through an angle of about 30°. One half of the remaining straight portion of each rod was removed by milling, leaving flat surfaces, *B*, as shown. With a strip of reflecting aluminum foil, *C*, between them the two surfaces were cemented together by an adhesive made from some of the resin dissolved in equal parts of carbon tetrachloride and chloroform. A strong bond and excellent internal reflection at the surface of the bond were thus obtained.

To prevent stray light from entering the lateral surfaces of the instrument, it became necessary to coat these with an opaque lacquer which still allowed internal reflection to take place. A coating made from aluminum powder dispersed in the above-mentioned resin solution was satisfactory. Flat surfaces, perpendicular to the resin rod at each point, were ground with fine emery at *D*, where the light enters the comparator, and at *E*, the eyepiece, where it emerges. A final polish on these surfaces was obtained with a finely divided abrasive metal polish.

A small convex lens in a brass tube (not shown) was fitted to the eyepiece of the comparator. By focusing this lens upon the resin surface, *E*, the two half-circle fields appeared to be uniformly illuminated with diffused light. In using the comparator the usual care was taken to provide illumination of equal intensity upon both colored surfaces.

An angle of 60° between incident light sources was the only one tried and is not necessarily the maximum practical angle for a comparator of this type.



Determination of Copper in Plant Materials

Using the Dropping Mercury Electrode

J. FIELDING REED AND RALPH W. CUMMINGS
Department of Agronomy, Cornell University, Ithaca, N. Y.

THE use of the dropping mercury electrode in analysis for copper has received some attention and should be adaptable to the determination of copper in plant material and in soils. The earlier investigations of Shikata (*8*), Mandi (*3*), and Roncato and Bassani (*7*) were of a preliminary nature, did not include limits of copper or of interfering materials, and were not conclusive. Thanheiser and Maassen (*11*) reported the polarograph to be very useful in analysis of steel for copper in the presence of other metals and Hohn (*2*) in his monograph included an outline for copper determination in brass and metal alloys. Suchy (*10*) used the dropping mercury electrode for simultaneous estimation of copper, bismuth, lead, and cadmium, and Stout (*9*) suggested a procedure for polarographic estimation of copper.

In this investigation the regulating solutions suggested by the above authors were tried in the presence of any interfering materials that might be present in plant materials and a method was worked out for the polarographic determination of copper in plant materials.

Tests of Regulating Solutions

Since ferric iron interferes with the determination of copper, it must be eliminated quantitatively from solution. This may be done with inappreciable loss of copper by adding an excess of ammonium hydroxide to the boiling solution and filtering. In testing the suitability of the various regulating solutions for copper, direct polarographic determinations were made of copper (as copper sulfate pentahydrate) added to these solutions. Then, in addition, copper standards were acidified with sulfuric acid and carried through the ammonium hydroxide treatment, the filtrates were evaporated to dryness, and the residues were taken up in the regulating solutions and polarographed.

Stout's solution of ammonium acetate–tartaric acid and Hohn's Grundlösung A were eliminated as giving unsatisfactory curves. In neither case could the curves be repeated with any quantitative precision. Hohn's Grundlösung C is a strongly ammoniacal solution of ammonium chloride containing methyl cellulose as a stabilizing colloid. In order to pass nitrogen through this solution to free it of oxygen it was necessary to add caprylic alcohol to prevent foaming over (*5*). Although there is some irregularity in the curves produced in this solution, it is possible to use it for higher concentrations of copper. At concentrations below 4 micrograms of copper per ml. of solution, the curves were unsatisfactory because the linear spread of the copper "break" (along the voltage axis) was so great as to make measurement difficult where quantities of copper are small and the wave height is correspondingly small.

Suchy (*10*) recommends as a regulating solution a 10 per cent solution of sodium potassium tartrate. This solution is alkaline owing to hydrolysis. Other solutions used by Suchy were acid and alkaline solutions of sodium citrate and sodium tartrate. All these solutions of Suchy's were tried with various copper concentrations. Where the copper solution was acidified with sulfuric acid, neutralized with an excess of ammonium hydroxide, filtered, washed, evaporated to dryness, and taken up in the various solutions of Suchy, free ammonia was liberated on addition of any of the alkaline solutions and unsatisfactory curves resulted.

The most satisfactory curves were obtained with acid sodium citrate (pH 3.8 to 4.2), made by mixing equal quantities of 0.5 *M* sodium hydroxide and 0.5 *M* citric acid. It was comparatively easy to free this solution of dissolved oxygen, requiring 10 to 20 minutes of bubbling in pure nitrogen, whereas the alkaline solutions required 60 to 80 minutes. Where copper alone was added to the regulating solution and polarographed (without the acidification, neutralization, filtration, etc.) there was a tendency toward a slight maximum. This was overcome by the addition of 1.0 ml. of 0.05 per cent acid fuchsin, $C_{20}H_{17}N_3(SO_2ONa)_2$, to every 10 ml. of regulating solution.

Table I. Base Mixture

Material	Element	Mg.	% in Plant[a]	Material	Element	Mg.	% in Plant[a]
KCl	K	10.0	1.0	$MnSO_4$	Mn	0.2	0.02
Na_2CO_3	Na	1.0	0.1	$NiSO_4.7H_2O$	Ni	0.05	0.005
$CaCl_2$	Ca	10.0	1.0	$CoCl_2.6H_2O$	Co	0.05	0.005
$MgSO_4.7H_2O$	Mg	5.0	0.5	$PbCl_2$	Pb	0.05	0.005
H_3PO_4	P	2.0	0.2	$CdCl_2.2H_2O$	Cd	0.05	0.005
$FeCl_3.6H_2O$	Fe	1.0	0.1	$ZnSO_4.7H_2O$	Zn	0 05	0 005
$Al_2Cl_6.12H_2O$	Al	0.2	0.02	$CuSO_4.5H_2O$	Cu	0.05	0.005
SiO_2 (silicic acid)	Si	20.0	2.0	As_2O_5	As	0.05	0.005

[a] Assuming 1.0-gram plant sample represented in 10-ml. solution.

Interfering Materials

To determine the concentration limits of materials other than copper which may occur in the plant ash without interfering with the determination of copper, solutions were made up containing all of those cations or anions that are likely to appear in an ashed sample. The basic synthetic solution which approximates plant ash is listed in Table I.

Now a series of solutions was prepared in which each con-

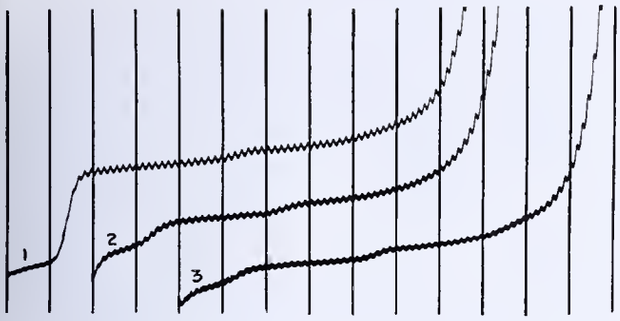


FIGURE 1. RECOVERY OF COPPER ADDED TO PLANT MATERIAL

1, 2, 3. Ashing methods referred to in text. Sensitivity 1/30, drop rate 2.5 seconds, distance between abscissas 0.15 volt

stituent was varied in concentration, holding the other constituents constant. The solutions were then treated with ammonium hydroxide, filtered, washed, and evaporated to dryness, the residue was taken up in the acid sodium citrate, fuchsin was added, and copper was determined polarographically.

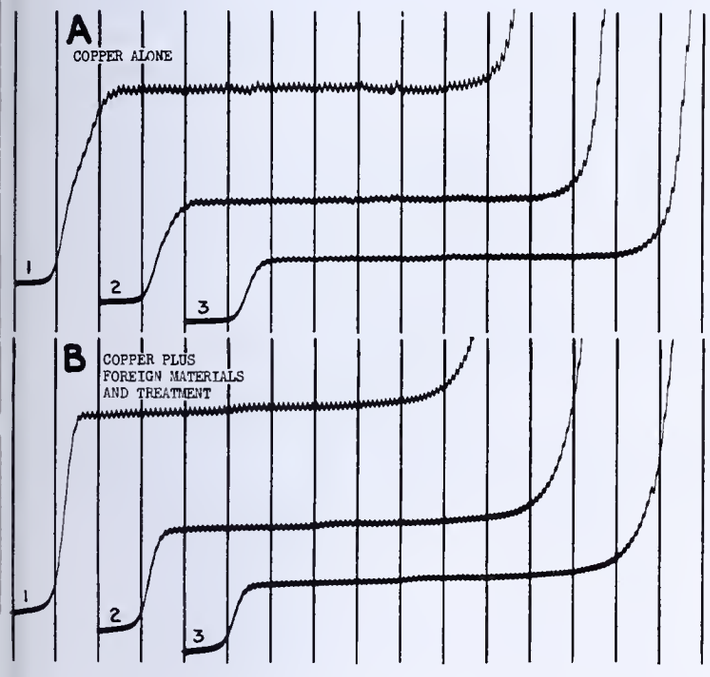


FIGURE 2. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT

Sensitivity 1/150, drop rate 2.5 seconds, distance between abscissas 0.15 volt

When the copper concentration was maintained at 5 micrograms per ml. (assuming 1.0 gram of plant material ashed and taken up in 10 ml. of regulating solution, this is equivalent to 0.005 per cent copper), there was no interference from any of the materials considered even when present at concentrations ten times as great as listed in Table I. From this it was evident that there would be no interference from any of these elements at concentrations that greatly exceed their normal concentration in plant material.

The anode potential was measured against the saturated calomel half-cell so as to correct the half-wave potential for it. There was little variation in anode potential and this operation might well be omitted in routine determinations. In the regulating solution used, the corrected half-wave potential for copper was −0.15 volt.

Ashing Procedure

After the method proved satisfactory for copper determinations in synthetic plant ash, a number of different plant materials were ashed in the following ways and copper was determined polarographically by the method used with the synthetic ash:

1. A wet-ashing procedure involving nitric, sulfuric, and perchloric acids.
2. Ignition in a muffle furnace at 450° C. for 12 to 16 hours.
3. Addition of nitric acid followed by ignition at 450° C. for 12 to 16 hours, addition of nitric acid again followed by reignition in the muffle.
4. Ignition in a muffle furnace at 650° C. for 12 to 16 hours.

To determine the effectiveness of the ashing procedures for recovery of added copper, 160 micrograms of copper as $CuSO_4 \cdot 5H_2O$ were added to the plant materials and the above ashing procedures were repeated. In all the dry-ashing trials, separate samples after ignition were taken up independently in hydrochloric and in sulfuric acid. In only the wet-ashing procedure was added copper recovered. This supports the conclusions presented by Mitchell (*4*) with regard to the determination of traces of copper. In Figure 1 are given curves illustrating the ineffectiveness of two dry-ashing procedures commonly used. A blank was run on the reagents used in the wet-ashing procedure to see what part of the "recovered" copper was in the reagents themselves. The acids carried through the procedure and taken up in regulating solution gave 4 micrograms of copper, and the plant material with unadded copper gave 10 micrograms of copper. These are small in comparison with the amount added for recovery, 160 micrograms of copper.

Proposed Method

Place 0.5 to 2.0 grams of plant material in a 30-ml. Kjeldahl flask. Add 5 ml. of concentrated nitric acid, heat until brown fumes are evolved, add 1 ml. of concentrated sulfuric acid, and heat until charring begins, all the nitric acid being driven off. Add 1 to 2 ml. of 60 per cent perchloric acid and continue heating until the solution is colorless or a pale yellow and the excess perchloric acid is driven off. Dilute to 15 to 20 ml., heat to boiling, add a slight excess of concentrated ammonium hydroxide, boil for a minute, filter, and wash with slightly ammoniacal water. Evaporate the filtrate to dryness, take up the residue in 9 ml. of acid sodium citrate (made by mixing equal quantities of 0.5 *M* sodium hydroxide and 0.5 *M* citric acid), add 1 ml. of 0.05 per cent acid fuchsin, and determine the copper polaro-

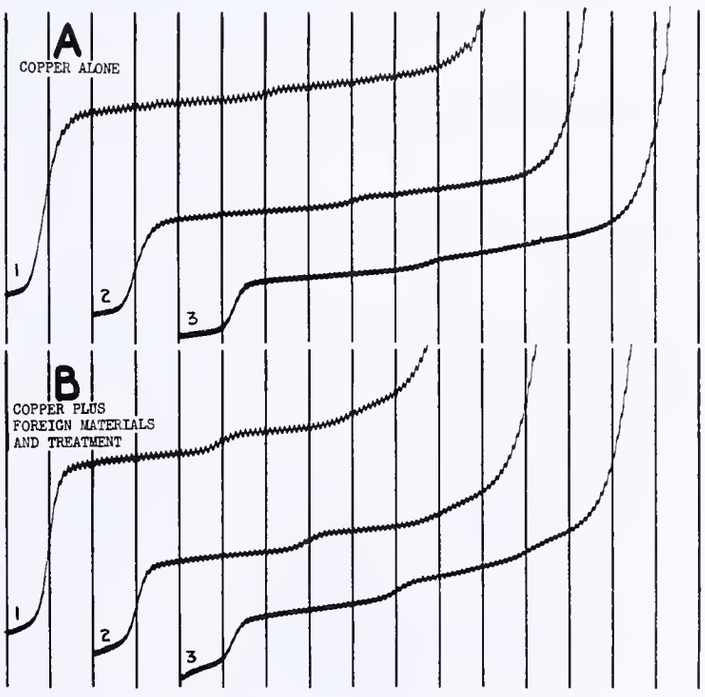


FIGURE 3. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT

Sensitivity 1/30, drop rate 2.5 seconds, distance between abscissas 0.15 volt

Table II. Comparison of Copper Alone and Copper Plus Foreign Materials and Treatment

	Copper Added γ/ml.	Galvanometer Sensitivity	Wave Height, Cu Alone Mm.	Corrected Cu Concentration, Cu Added + Blank γ/ml.	Wave Height, Cu + Interfering Materials	Corrected Cu Concentration, Cu Added + Blank γ/ml.
Figure 2	200	1/150	50.0	200	50.0	200
	100	1/150	26.0	100	25.0	100
	64	1/150	15.5	64	16.0	64
Figure 3	32	1/30	43.5	32	42.0	32
	16	1/30	22.0	16	21.0	16
	8	1/30	12.0	8.3	11.0	8.4
Figure 4	4	1/10	21.0	4.3	20.0	4.4
	1	1/10	9.5	1.3	10.5	1.4
	0.5	1/10	5.5	0.84	5.5	0.87
Figure 5	0.5	1/5	12.0	0.84	11.0	0.87
	0.2	1/5	9.5	0.54	8.5	0.57
	Blank	1/5	6.0	0.34	5.5	0.37

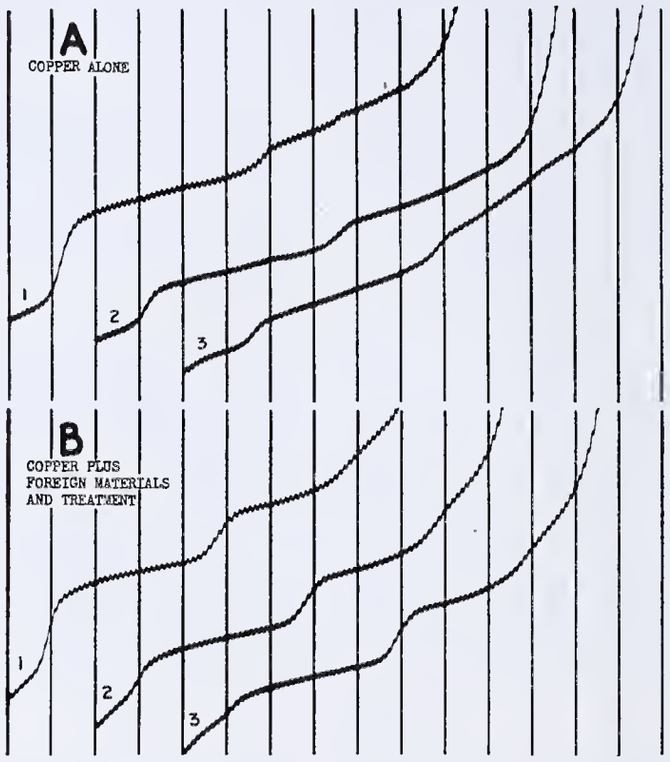


Figure 4. Comparison of Copper Alone and Copper Plus Foreign Materials and Treatment
Sensitivity 1/10, drop rate 2.5 seconds, distance between abscissas 0.15 volt

graphically. Oxygen is removed from the solution prior to the determination by bubbling in pure nitrogen.

Limits of Method

In order to determine the range of copper concentration for which the method is applicable, synthetic solutions were again made up which approximated ashed plant materials and whose composition was the same as that given in Table I. In this case the copper content of these solutions was varied, and they were analyzed for copper by the procedure suggested. The polarograms obtained were compared with others obtained by adding similar concentrations of copper to the sodium citrate solution, adding fuchsin, and polarographing. The equipment used in obtaining the current-voltage curves was the same as that described by the authors in a previous paper (*6*). The results of these determinations are indicated in Figures 2 to 5 and summarized in Table II.

The measurements of wave height were made by the so-called "intersection point" method denoted as method C by Borcherdt *et al.* (*1*). The accuracy, if several measurements are made and averaged, is about 0.5 mm. This obviously means that a greater percentage error is involved where the wave heights are small. The limits of the method were from 200 to 0.2 microgram of copper per ml. Expressed on a plant basis, if the procedure were carried out as outlined, using 1 gram of plant material and taking up in 10 ml. of regulating solution, this would amount to a range of from 0.2 to 0.0002 per cent of copper in the plant. The upper limit could be extended by using a lower galvanometer sensitivity or by diluting the solution.

Even in the case of the copper alone the "blank" was as high as 0.37 microgram per ml. It is evident that a blank is necessary for correction purposes and that with a blank of this magnitude it would not be possible to determine concentrations less than this amount. In order to avoid extra trouble in redistilling water and special purification of reagents, it would probably be better to select a 2-gram sample of plant material where unusually small amounts of copper are expected.

Plant Materials

To illustrate the effectiveness of the method on plants somewhat higher in copper, analyses were made of lettuce, the seeds of which had been pretreated with copper. The polarograms for these plants are shown in Figure 6. Dry-ashing methods gave less than 50 per cent of the copper found by wet-ashing these plants.

Summary

A method for the determination of copper in plant materials is proposed which involves the use of the dropping mer-

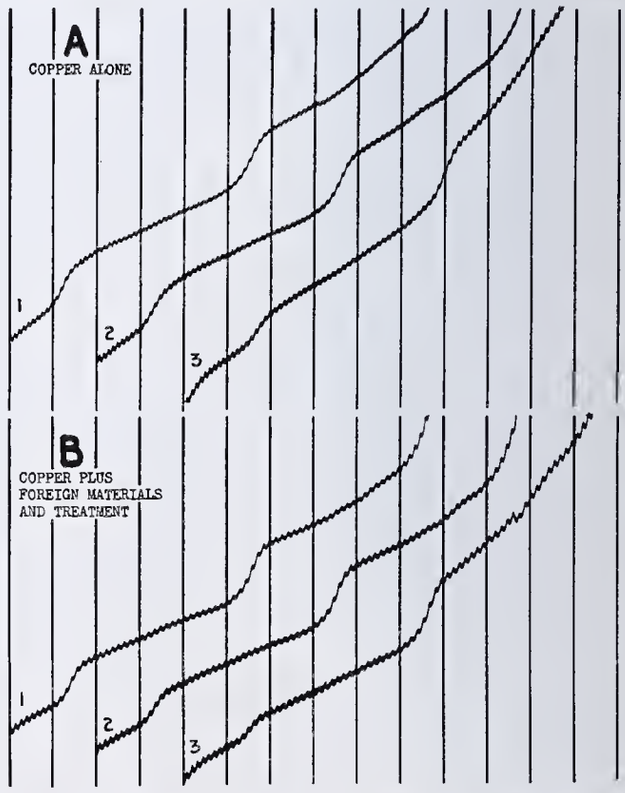


Figure 5. Comparison of Copper Alone and Copper Plus Foreign Materials and Treatment
Sensitivity 1/5, drop rate 2.5 seconds, distance between abscissas 0.15 volt

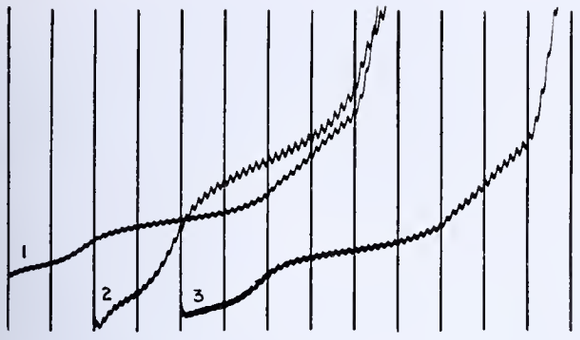


FIGURE 6. COPPER IN LETTUCE
(1) Sensitivity 1/30, 0.004% Cu; (2) sensitivity 1/10, 0.003% Cu; (3) sensitivity 1/30, 0.006% Cu. Drop rate 2.5 seconds, distance between abscissas 0.15 volt

cury electrode. Copper is determined in the presence of all the constituents ordinarily present in plant ash except those that are removed by addition of a slight excess of ammonium hydroxide. No interference is offered by any of the cations or anions likely to be found in plant ash even when present in comparatively large quantities.

Copper added to plant materials as copper sulfate could not be recovered by any of the dry-ashing methods used. A wet-ashing procedure was adopted involving final solution in sulfuric acid.

Limits of the method using a 1-gram sample of plant material are from 0.2 per cent or greater to 0.0002 per cent of copper in the plant.

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Systematic Qualitative Organic Microanalysis

Comparative Study of Procedures of Microextraction

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EXTRACTION procedures constitute an important step in the scheme of organic analysis on a micro scale (*1, 2*). These procedures are of two types: the removal of an active ingredient from a heterogeneous mixture and the removal of undesirable material from an impure product. Separation of the active principle from a crude drug mixture is an example of the first type; the preparation of derivatives and their consequent purification, an example of the second.

In control laboratories, where time is limited, methods which require less sample and consequently less extraction time are of distinct advantage, provided that the results are comparable to those obtained when using conventional apparatus. This investigation includes only methods for extraction of solid substances by heated solvents and excludes liquid-liquid extraction processes. The merits of a standard macro-Soxhlet, a semimicro-Soxhlet extractor and representative microextractors are compared.

Several microextractors, developed in recent years, were constructed for special investigations. The authors are not cognizant of any study dealing with the use of an extractor for general purposes involving the quantitative recovery of both extractive and residue.

The two types of extraction apparatus are siphoning and percolating; these may be of simple or complex design.

The macro-Soxhlet, its semimicro counterpart (commercially listed as "micro" Soxhlet extractor), the Colegrave (*7*), and the Wasitzky (*21*) extractors belong to the siphoning group. Extractors using the principle of percolation have been developed by Titus and Meloche (*20*), Gorbach (*13*), Hetterich (*14*), and Slotta.

Slotta's extractor (*18*), which is not described in the literature, is shown in Figure 4. A glass crucible with sintered-glass bottom holds the material to be extracted. Crucibles of different porosities may be substituted to correspond with the particle size of the substance and surface tension of the solvent. A Dimroth condenser reduces the over-all height of the apparatus. The receiving flask is connected to the extraction chamber by a ground-glass joint; an apparent disadvantage is the weight of this flask, approximately 15 grams, which reduces the accuracy when very small amounts of extracted material are weighed. When solution is transferred there is always the added risk of accidental loss. Two glass inserts of 5- and 10-ml. capacity, respectively, together with a distribution tube, serve for the extraction of small amounts of liquid.

Because of similarity of design to one or another of the microextractors mentioned, the apparatus of Blount (*5*), Browning (*6*), Fulton (*10*), Garner (*11*), and Gettens (*12*) were not included in this investigation.

Factors Influencing Extraction

SOLVENTS. Solvents were selected so that volatile and moderately volatile, low and high boiling, low and high surface tension menstrua were included. A solvent having special affinity for an extractable material likely to be present is preferable. The influence of atmospheric moisture on the solvent is reduced by using drying tubes on top of the condensers.

The range of boiling points of solvents tried was between 35° and 100° C. at normal pressure. The boiling point in a completely enclosed extracting system, or one with a comparatively small condenser, may be slightly higher, especially in the case of a very volatile liquid. Decomposition of natural products when extracted with common solvents may occur if the boiling points, at atmospheric conditions, are high. These two dangers may be avoided by the application of reduced pressure in the extraction apparatus (*13, 20, 21*).

The surface tension is another influence in obtaining a smooth-running extraction. The range of surface tensions encountered in common solvents for liquid-air interface, ex-

A B C D

FIGURE 1. COMPARISON OF METHODS OF EVAPORATION
A. Heating from underneath
B. Heating from above, with Chromolox ring
C. Spontaneous evaporation
D. Heating from underneath but removing vapor phase by a jet of air

pressed in dynes per cm., includes: ethyl ether 20° C. = 17, 35° C. = 15; ethyl alcohol 20° C. = 22, 70° C. = 18; and water 20° C. = 73, 100° C. = 59. In the lower range of surface tensions the effect is mainly that of penetrability of the solid; Lehrecke (*16*) states that the penetrability of an organic solvent into a solid is inversely proportional to the surface tension. In the higher range, difficulties with the refluxing liquid are encountered.

SYSTEMS STUDIED. Salicylic acid and kieselguhr extracted with ether, anthracene and starch extracted with ether, and sodium chloride and aluminum oxide extracted with water, were the mixtures and solvents tested in comparing the macro- and semimicroextractors. Because of mechanical difficulties, salicylic acid mixed with sand and extracted with ether, caffeine and barium sulfate extracted with alcohol, and sodium chloride and sand extracted with water, were used for comparing the microextractors. The salicylic acid–sand mixture was not used in the macroapparatus because of the great variation in bulk, which was appreciable but less noticeable when used in the smaller extractors.

All the mixtures were tested in various ratios, so as to have the solid components approximately equal in weight, the soluble part in excess, and the insoluble portion in excess. The last mentioned would correspond to the extraction of material in which the percentage of soluble matter is small (towards the lower limits of the weighing range of a microchemical balance).

Container for Sample

To compare the merits of the materials from which containers are commonly constructed, filter paper in the form of dishes, disks, and thimbles; Alundum thimbles; vessels with sintered glass bottoms; and platinum as well as Monel metal containers were studied.

It is very difficult to obtain constant weight on large masses of filter paper owing to its hygroscopicity. By using standardized procedures (*15*) or certain precautions to prevent access of moisture, the weights of paper thimbles can be reproduced within the limits of accuracy of either method, but these procedures are very cumbersome. In the majority of cases, this difficulty in obtaining constant weights of the filter paper used in some extractors prevented accurate determination of the residue. Even though the filter paper thimbles were subjected to a preliminary extraction, fibers were carried over during the extraction proper and had to be removed by centrifuging—a step that involves two unnecessary transfers.

Although it was possible to obtain constant weight with filter thimbles of Alundum, they were unsatisfactory for use with the apparatus on hand because of the retention of solvent within the thimble, which resulted in overflowing. The use of crucibles with sintered glass bottoms has been advocated in some microextractors—e. g., Slotta's and Browning's (*6*)—and constancy of weight has been reported, provided definite weighing conditions are maintained. From experience with similar apparatus in quantitative microanalysis, it can be deduced that the weight constancy will certainly remain within ±20 micrograms.

From the analytical viewpoint platinum appears to be the most satisfactory material for thimbles or dishes, since it is inert to most solvents, can attain constant weight quickly, and is readily obtainable in any desired form. A possible objection is its initial cost, although if it is utilized over a long period of time the cost is well distributed, for it seldom needs replacing. Preliminary experiments indicate that other metals may be substituted, but they lack the general applicability of platinum.

POROSITY. Conflicting statements have been made regarding the suitable porosity of sintered glass plates when the solvent is removed by gravity. Blount (*5*) recommends G_2, while Browning (*6*) suggests G_3.

Tests conducted in the present investigation, using Jena products, showed that sintered glass plates of porosity G_3 were satisfactory for use with alcohol, while G_4 filters were not sufficiently fast if accumulation of solvent was to be avoided (for porosity gradings see Prausnitz, *17*). The porosities as listed by Ace Glass, Incorporated, would correspond as follows: Jena O to Ace A; 1 to B; 2 to C; 3 to D; and 4 to —.

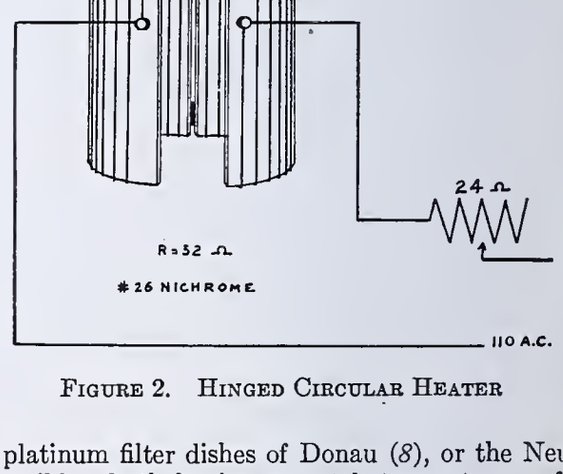

FIGURE 2. HINGED CIRCULAR HEATER

The platinum filter dishes of Donau (*8*), or the Neubauer filter crucibles, both having a mat between two perforated platinum surfaces, may be made up by adjusting the thickness of the mat to suit the conditions of the experiment.

TIME REQUIRED FOR COMPLETE EXTRACTION. The time required for the exhaustion of the extractable material from the sample is best determined by successive extractions until the weight of the last flask substituted shows no further increase.

Another method, frequently used in alkaloidal assaying, consists of removing a little of the freshly siphoned or percolated solution and testing it with the proper reagent until negative results are obtained.

In order to distribute the solvent evenly upon the surface of the sample and prevent channeling, Prausnitz (*17*) constructed the "icicle crown" condenser tip, which was later incorporated in Gorbach's extractor (*13*). The more effective distribution of the condensate results in a shorter extraction time.

Special Procedures and Apparatus Developed

EVAPORATION OF SOLVENT AFTER EXTRACTION. To distill off the solvent and at the same time prevent creeping of the solid

material, the vapors above the surface of the liquid are removed by blowing air onto it or by applying heat from the top (*1*, *4*). In the latter method, a Chromolox resistance ring of 150 watts (outer diameter 65 mm., inner diameter 25 mm., and 5 mm. thick) is placed around the upper part of the vessel and gradually lowered as evaporation proceeds.

A comparison of these methods, with the usual one of heating from underneath, is presented in Figure 1, corresponding receiving flasks containing equal amounts of ether and of salicylic acid were used. The picture, taken with a polaroid screen to remove reflected highlights, illustrates clearly the advantages and disadvantages of the methods applied.

Hinged Circular Heater. The condensation of solvent in the ground-glass joints of most of the extractors examined not only resulted in a direct loss, but was also a source of annoyance because of the suction formed when some of the solvent in the joints evaporated. To overcome this, Titus and Meloche (*20*) warmed the joint of their apparatus by winding resistance wire around it and heating by passage of an electric current. To avoid winding all the glass joints, a generally applicable device was constructed (Figure 2). The outside, circular, hinged electric heater consists of a hollow cylinder of hardened asbestos cement, cut in halves in the axial plane and with halves hinged together. Each half of the cylinder is wound vertically with 300 cm. (10 feet) of No. 26 B. and S. Nichrome wire having a resistance of 2.61 ohms per 30 cm. (1 foot), with one of the hinges serving as a contact. A slide-wire resistance of 24 ohms and 3.3 amperes is placed in series with the heater, so that the amount of heat radiated may be controlled. The hinge allows observation of the rate of boiling and permits the heater to be removed from the extractor simply by disconnecting one of the contacts leading to the external wiring.

In experiments using water as a solvent it was extremely difficult to prevent excessive condensation on the walls of the outer shell (as in Titus and Meloche's and Gorbach's apparatus) and on the inner walls of the reservoir in Colegrave's and Wasitzky's extractors. In one experiment some of the condensed solvent dripped onto the hot shell of the Gorbach apparatus and cracked it. The application of the external heater prevented recurrence of this trouble and since its introduction boiling and vaporization have appeared to run smoothly and condensation has been regular.

Modifications of Original Design

Slight modifications were introduced into some of the original designs for the purpose of facilitating certain manipulations.

The ground-glass joint of the thimble in Titus and Meloche's (*20*) extractor was reversed—i. e., the lower portion was made the outside member, so that the filter paper disk would form an upright dish and avoid loss of inert material when removed (Figure 5).

Loss of volatile solvent from the original extractor of Colegrave (*7*) suggested the introduction of a ground-glass joint at the junction of the condenser and the top of the chamber. This modified apparatus (Figure 4) was found more satisfactory in preventing solvent losses. A small glass receiver, placed in the bottom of the chamber, made unnecessary the transfer of solution for evaporation and weighing. A similar device was used for Wasitzky's extractor (*21*).

Experimental

The salicylic acid, sodium chloride, and sand used were Merck's Blue Label reagents which required no purification, but the anthracene and caffeine had to be recrystallized. For precautionary measures the solid insoluble components, kieselguhr, barium sulfate, sand, and starch, were previously boiled in some of the solvent selected for the extraction, so that any solvent-soluble material would be removed.

The organic solvents, ethyl ether and ethyl alcohol, were distilled until the boiling point of each was sufficiently constant. Only those tests for purity (*22*) were performed which would yield information about the solvent properties. The water used was distilled by means of a laboratory still and protected from atmospheric carbon dioxide.

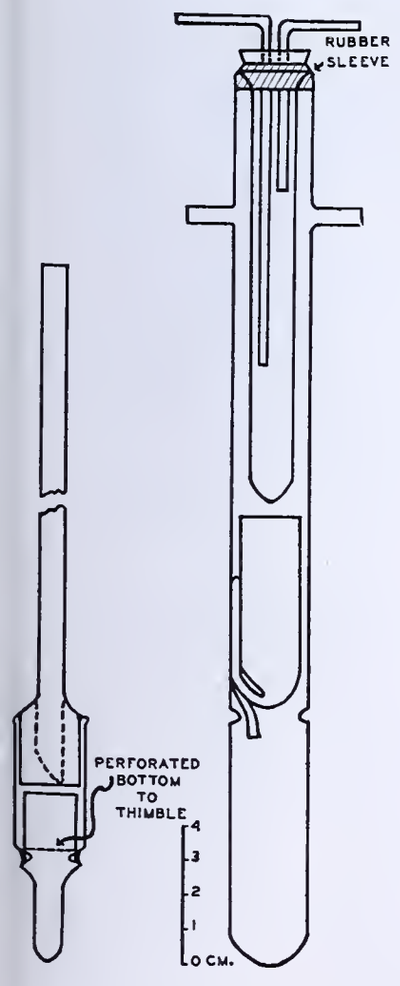

Figure 3. Microextractors
Left. Hetterich type
Right. Wasitzky type

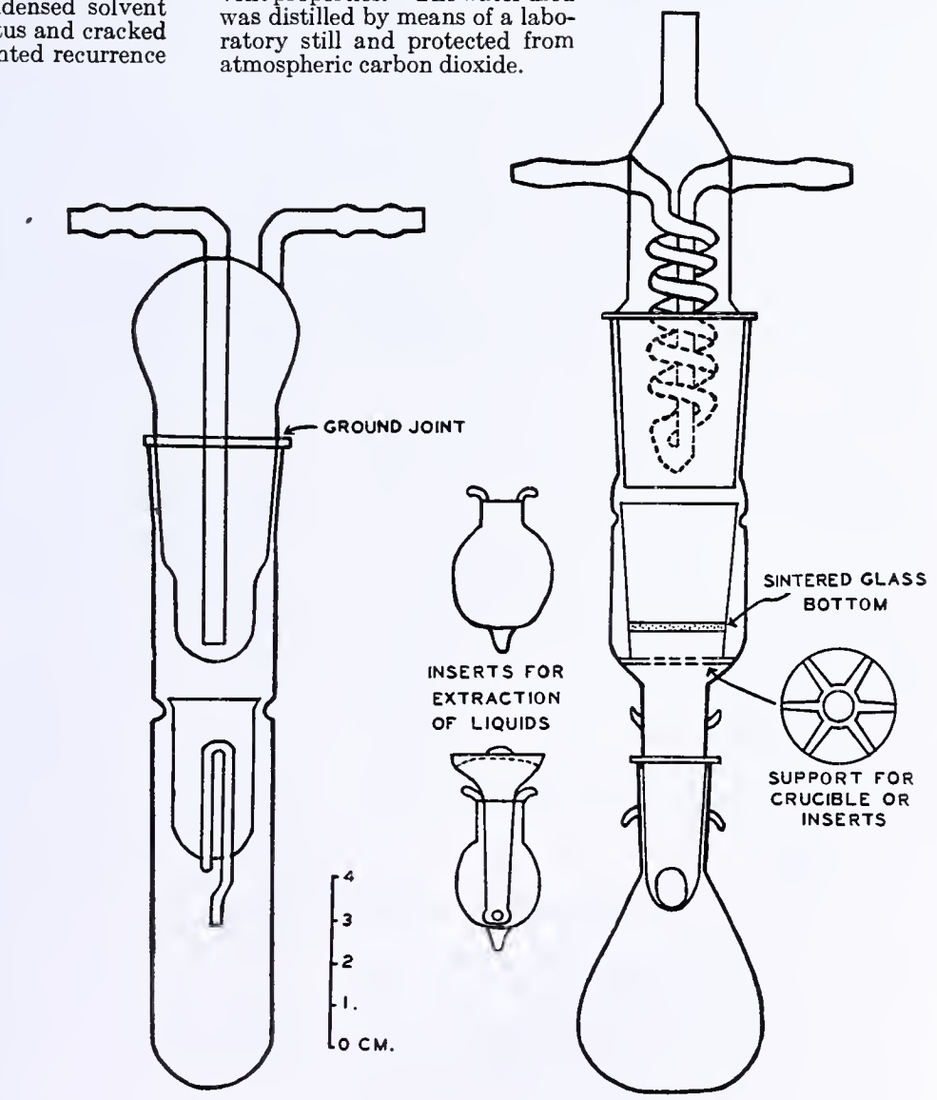

Figure 4. Microextractors
Left. Modified Colegrave type
Right. Slotta type

Table I. Comparison of Volume Measurements in Semimicro- and Microextractors

(Surface tensions of solvents used, dynes per cm.: ethyl ether: 20° C. = 17, 35° C. = 15; ethyl alcohol: 20° C. = 22, 70° C. = 18; water: 20° C. = 73, 100° C. = 59)

Apparatus	Ratio of Volumes: Volume of whole chamber to initial volume of liquid, ml.	Volume retained in extraction thimble before removal to initial volume of liquid, ml.	Volume of whole chamber to volume retained in extraction thimble before removal, ml.	Contact
Semimicro-Soxhlet	25:20 = 1.2	8:20 = 0.4	3	Long
Titus and Meloche	125:5 = 25	0.2:5 = 0.04	625	Variable
Gorbach	55:2 = 28	0.2:2 = 0.1	280	Short
Colegrave (improved)	60:10 = 6	4:10 = 0.4	15	Long
Wasitzky	60:10 = 6	2.5:10 = 0.25	24	Long
Hetterich	9:1 = 9	0.2:1 = 0.2	45	Medium
Slotta	50:20 = 2.5	0.5:20 = 0.03	100	Variable

Procedure for Microextractions. Each flask, extraction or weighing, is tared with an object of similar material—i. e., a Pyrex flask with a Pyrex beaker and objects of soft glass with soft glass flasks of approximately the same size and shape. The Pyrex flasks for the macro- and semimicro-Soxhlet extractors are carefully tested for electrostatic charges (*19*); otherwise constancy cannot be obtained for the initial weighing. The receiving vessels for the microapparatus are made of either soft or "Jena Geräte" glass. The ingredients for the centigram and milligram extraction processes are weighed separately to obtain the exact weight of each component present. The charging tubes with ground caps (*3*) are employed for the direct introduction of the solid constituents and all weighings are made on a microchemical balance. The weighing flasks, after thorough cleaning and drying, are placed in the balance case to come to temperature equilibrium and then weighed under the same conditions as described below for the extract.

After weighing the sample and flask, the required amount of solvent is pipetted into the flask and the latter is set in the proper position, so that movements within the shell are minimized.

The soluble and insoluble materials are placed in the thimble and gently mixed with a glass rod which is later rinsed with a little of the solvent. Besides washing off any sample that may adhere to the rod, the few drops of solvent moisten the sample and filter paper, thus preventing splashing when the first drop or two of solvent fall from the condenser tip.

A maximum period of one hour is usually adequate to exhaust the soluble portion from the mixture in each microextractor. Only if it is found that insufficient extract (as calculated from the original sample taken) is obtained should a longer extraction time be used.

After completion of the extraction the apparatus is dismantled. Where a very volatile solvent is used, it is advisable to wash off the tip of the siphon, funnel, or thimble with a few drops of solvent. The receiving flasks of the apparatus are removed and evaporation of solvent is commenced. After apparent dryness is reached, each flask is put into the drying oven for 5 minutes at a temperature of 5° C. above the boiling point of the solvent used. The flasks are then placed in turn for 10 minutes each on a Petri dish and in the balance case to attain temperature equilibrium, and weighed after 25 minutes from the time of removal from the oven. All transfers from one place to another are made with forceps.

The amount of extract found is compared with the amount of soluble material taken and the percentage of error of recovery calculated from these figures; the error is not calculated from the total amount of sample taken.

The details of construction of the microextractors used are shown in Figures 3, 4, and 5, drawn to relative size.

Table I shows the ratios of important volume measurements of apparatus and solvents in the extraction process. The value of "short" in column 5 corresponds to an average interval of less than 1 minute and "long" to about 4 or more minutes. From Table I, and from experience in many extraction procedures, the following generalized conclusions may be drawn.

The greater the ratio of the volume of the whole chamber to the initial volume of liquid, the less satisfactory is the extractor for use with high-boiling solvents with high surface tensions.

The smaller (within practical limits) the ratio of volume retained in the extraction thimble before removal to initial volume of liquid, the more satisfactory the extraction process becomes, with regard to the quantitative recovery, especially in the case of the extraction of slightly soluble substances.

The value of the ratio of the volume of the whole chamber to the volume retained in the extraction thimble before removal is dependent upon the method of removal of solution; for percolation the differences are not very significant, whereas for the siphoning method the different values assume importance.

As observed in the extractors of Hetterich and Gorbach, the volume of the reservoir is so small that when evaporation takes place and condensate of liquid of higher surface tension (as water) forms on the inside of the extraction chamber, very little solvent remains in the reservoir.

Comparison of Macro- and Semimicro-Soxhlet Apparatus

Table II shows the results obtained when using a semimicro-Soxhlet extractor; they are at least equivalent to those of the conventional macro-Soxhlet extractor. In addition, however, important advantages are offered by the use of the smaller apparatus: The period of extraction is shorter, up to two thirds of the time for moderately soluble substances, although with a very soluble constituent mixed with an excess of inert material the time of complete extraction remains the same for both macro- and semimicroprocedures; less material is required; less space is occupied; no additional manipulations are introduced; and the initial cost is very favorable. Based upon these advantages, the selection of the semimicro-Soxhlet extractor instead of the macroapparatus as the standard for the following investigations is justified.

The accuracy of the macro-Soxhlet extractor, ±1.9 per cent, was computed from 25 determinations with varying solvents, mixtures, and ratios of ingredients, and calculated

Table II. Results Obtained with Macro- and Semimicro-Soxhlet Extractors

Components and Solvents	Ratio[a]	Macroextractor: Weight of Sample: Total	Macroextractor: Active ingredient	Macroextractor: Recovery	Semimicroextractor: Weight of Sample: Total	Semimicroextractor: Active ingredient	Semimicroextractor: Recovery
		Grams	*Gram*	%	*Gram*	*Gram*	%
Salicylic acid–kieselguhr with ether	1:9	1.0000	0.1000	97.0	0.2004	0.0200	100.5
		1.8020	0.1802	99.5	0.2036	0.0204	101.5
		1.5002	0.1500	99.5	0.1982	0.0198	100.5
		1.6290	0.1629	100.0	0.2012	0.0201	101.5
		2.0000	0.2000	96.5	0.2112	0.0211	99.0
		1.0054	0.1005	101.0	0.1980	0.0198	101.5
Salicylic acid–kieselguhr with alcohol	1:9	1.1207	0.1121	102.0	....	....	...
		1.0462	0.1046	100.0	....	....	...
		1.1315	0.1132	98.5	....	....	...
		1.0184	0.1018	102.5	....	....	...
Anthracene-starch with ether	1:9	1.0785	0.1079	103.0	0.2232	0.0223	99.5
		1.0519	0.1052	100.0	0.2072	0.0207	99.0
		1.0297	0.1030	98.5	0.2381	0.0238	99.0
		1.0686	0.1069	98.0	0.2570	0.0257	99.0
	1:1	1.0000	0.5000	99.5	0.2000	0.1000	99.5
		1.0004	0.5000	98.5	0.2004	0.1002	99.0
Sodium chloride–aluminum oxide with water	1:9	1.0005	0.1000	103.0	0.2183	0.0218	99.0
		1.0048	0.1005	100.0	0.2075	0.0208	99.5
		....	....	...	0.2068	0.0207	97.5
		....	....	...	0.2051	0.0205	102.5
	1:1	1.0000	0.5000	101.5	0.2022	0.1011	100.0
		1.0109	0.5050	95.0	0.2025	0.1013	97.5

[a] Parts of active ingredient to parts of inert ingredient.

TABLE III. COMPARISON OF MICROEXTRACTORS

(Experimental conditions: ratio of active to inert ingredient, 1:1; extraction time, 1 hour)

Components and Solvent	Semimicro-Soxhlet Extractable substance	Recovery	Titus and Meloche Extractable substance	Recovery	Gorbach Extractable substance	Recovery	Colegrave (Modified) Extractable substance	Recovery	Wasitzky Extractable substance	Recovery	Hetterich Extractable substance	Recovery
	Mg.	%	*Mg.*	%	*Mg.*	%	*Mg.*	%	*Mg.*	%	*Mg.*	%
Salicylic acid–sand with ether	15.93	102	16.78	94.0	25.26	94.5	12.66	103	9.70	106	13.17	99.0
	20.96	100	33.33	98.5	28.97	97.0	19.42	101	11.42	108	9.10	114
	24.58	98.5	0.42[a]	64.5	2.14[a]	94.0	0.71[b]	65	...	...	9.76	98.0
	...	...	11.94[c]	99.0	88.25[c]	100	61.31[d]	99.5	...	...	0.91[b]	98.0
Salicylic acid–sand with alcohol	...	...	11.33	100.5	2.97	101	...	...	...	...	...	...
	...	...	25.03	101	4.43	93.0	...	...	...	...	...	...
Caffeine–barium sulfate with alcohol	26.87	100.5	9.66	102	4.58	104	6.38	99.5	26.53	105	2.64	106
	15.24	99.0	9.06	102.5	7.42	100	8.99	99.0	21.23[e]	101	2.84	96.0
	18.54	101	1.06[a]	108	0.61[a]	127[d]	1.42[b]	105[e]	1.88[b]	102.5	1.18[b]	102.5
	...	...	40.94[c]	101	24.37[f]	99.5	43.76[f]	99.5	51.60[f]	101	10.78[f]	99.5
Sodium chloride–sand with water	23.83	100	14.02	100	11.63	99.0	9.97	99.5	...	...	4.37[g]	92.5
	21.46	102	10.57	100	9.81	101.5	11.48	103	...	...	5.74	74.0
	20.16	99.0	1.37[b]	99.0	2.08[b]	104[e]	1.53[b]	101	...	...	...	...
	...	...	24.78[f]	101	8.15[f]	102	96.51[f]	100	...	...	...	...

[a] Ratio 1:100. [b] Ratio 1:50. [c] Ratio 100:1. [d] High results probably due to mechanical carrying-over of inert material. [e] Solution centrifuged and transferred to fresh receiver. [f] Ratio 50:1. [g] Frequent bumping, probably due to shape of receiver.

for the amount extracted. Maximum single deviations from the theoretical values were +3.3 and −9.0 per cent (one case each). Average deviations from the mean value were +1.8 and −3.3 per cent. The accuracy of the semimicro-Soxhlet extractor, ±1.85 per cent, was calculated in the same way from 28 determinations. Maximum single deviations from the theoretical values were +5.9 and −6.7 per cent (one case each). Average deviations from the mean value were +2.1 and −1.7 per cent. Not all the values taken for this evaluation appear in Table II.

Microextractors

A further comparison is made between the semimicroextractor and the various microextractors in Table III, which is self-explanatory.

Similar experiments have been carried out for the ratios of active to inert ingredient: 100 to 1, 50 to 1, 10 to 1, 1 to 10, 1 to 50, and 1 to 100. On the basis of these observations, the following generalized conclusions can be drawn for each individual microextractor:

TITUS AND MELOCHE APPARATUS. Satisfactory for use when the solid components are equally proportioned, when the soluble material is in excess, and with high-boiling solvents.

GORBACH APPARATUS. Satisfactory for use when the solid components are equally proportioned, when the soluble material is in excess, and when the inert material is not too finely divided. This extractor is equipped with an "icicle" distribution crown which permits faster extraction. For liquids having a surface tension near that of water, this apparatus is not recommended.

COLEGRAVE APPARATUS (ORIGINAL AND IMPROVED FORM). Satisfactory for use when the solid components are equally proportioned, when the soluble portion is in excess, and when water is the solvent. This extractor is one of the least expensive to purchase.

WASITZKY APPARATUS. Satisfactory when the soluble material is in excess of the inert. In general, this extractor is similar to the improved Colegrave apparatus with ground-joint condenser and is superior to the simple one.

HETTERICH APPARATUS. Satisfactory for use when the soluble material is in excess and when the solvent is low boiling, but unsatisfactory in the present form for use with water as solvent.

From the values in Table III, one can deduce that the accuracy and precision of the determinations of the extract in the microextractors fall within the range calculated for the macro- and semimicro-Soxhlet extractors—i. e., ±2.0 per cent. Greater errors will occur as one approaches the unfavorable extremes for the microextractors studied; this investigation has suggested the limits of practicability and greater variations should be avoided. An accuracy of 0.75 per cent has been claimed for some microextractors (*9*), but will be obtained only in very special cases. The present investigation has clearly shown that, in general, ±2.0 per cent is a reasonable accuracy to expect.

The advantages of the microextractors, even over the semimicroapparatus, may be summarized as: a shorter total extraction time, the possibility of using smaller amounts of

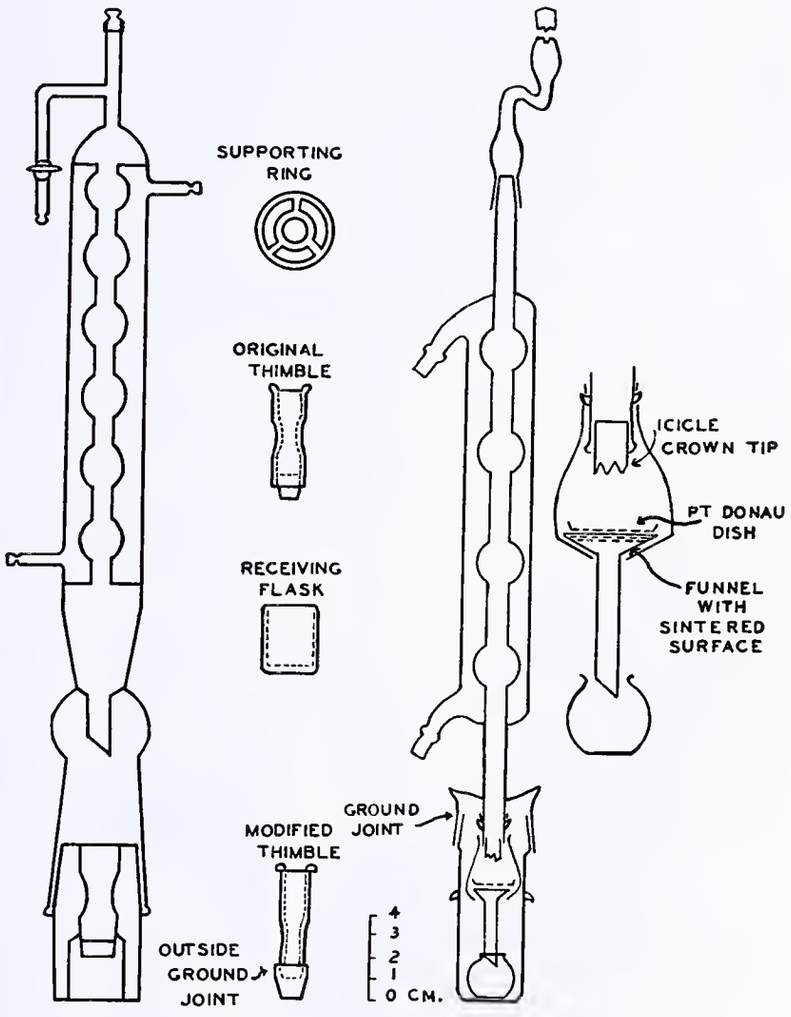

FIGURE 5. MICROEXTRACTORS
Left. Titus and Meloche type
Right. Gorbach type

material (limited only by the weighing accuracy of the available microchemical balance), and the comparative sturdiness of the apparatus. The less commonly used principle of percolation gives better service with respect to general applicability than siphoning (Soxhlet). The apparatus developed by Titus and Meloche is easier to manipulate than the one introduced by Gorbach. One feature, however, which is seldom applied in any of the known macro- or microextractors is incorporated in the Gorbach apparatus—namely, the use of a platinum filter dish as a container for the material to be extracted. Filter devices of metal permit the highest precision in weighing and maximum percentage of recovery of the residual matter without any contamination from the filtering material.

The weighing of milligram amounts of residue on comparatively large filter paper surfaces (extraction thimbles) will not give reliable results, and the microanalyst has to face the fact that all extractors of the siphoning type using paper thimbles are limited to the determination of the extractable matter.

The difficulties in weighing the residual material in the apparatus examined prevented the development of a general formula for the efficiency of an extraction process and of numerical terms for the evaluation of the various factors influencing the extraction.

To accomplish this, a satisfactory microextractor should use the principle of percolation of the solvent through the sample which is held in a platinum container provided with a filter layer of platinum-iridium sponge. The sample should be agitated by means of hot solvent vapors in order to increase the temperature, and condensation of the solvent effected by means of a short-stem water condenser having a device for distributing the refluxed solvent. For convenience the sample container should be attached to the condenser, with the latter connected to the outer shell by means of an outside ground joint. The receiver for the extract should be of low weight, preferably of platinum, and of larger top surface to allow fast evaporation from the top without loss due to creeping. Protection from the influence of atmospheric moisture must also be considered. The ratio of solvent volume to solvent in the thimble should be about 50 to 1, with the ratio of chamber volume to initial volume of solvent as small as practical. A circular hinged heater supplying uniform heat to all sides is recommended for the proposed extractor.

Acknowledgment

The authors express their thanks to Arthur Osol of the Philadelphia College of Pharmacy and Science for his constructive criticism and to Ellice McDonald, director of the Biochemical Research Foundation, for his interest and support throughout the work.

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Presented before the Division of Microchemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wis. Abstract of a portion of a thesis submitted to the faculty of the Philadelphia College of Pharmacy and Science by William G. Batt in partial fulfillment of the requirements for the D.Sc. degree.

Pressure Regulator for Microdetermination of Carbon and Hydrogen

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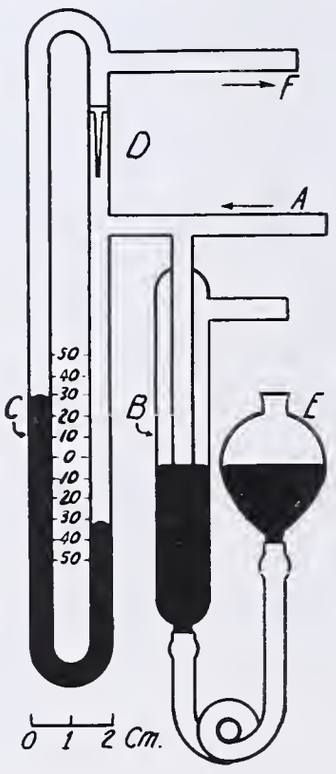
THE modified flowmeter described here has proved satisfactory as a pressure regulator for microcombustions. The chief advantages are that it avoids the use of a hydrostatic pressure head and permits a permanent adjustment of the pressure to be made, if desired. It is particularly suitable for undergraduate students taking their first course in analytical chemistry, and it has been used in this laboratory with success over a period of years.



The regulator is shown in the accompanying figure. The oxygen gas enters at *A* and, depending upon the height of mercury in the overflow tube, *B*, will exert a certain pressure on the capillary, *D*. This pressure is registered on the mercury manometer, *C*, which has a paper millimeter scale behind it. The rate of flow through *F* to the furnace depends upon the size of the capillary as well as upon the head of mercury. A convenient size for the capillary is 0.2 to 0.3 mm.; this will give a flow of 4 to 6 cc. per minute with an appropriate pressure.

The capillary may be drawn out, and the piece of glass tubing into which it is sealed held in the manometer by means of rubber tubing. The rate of flow is then measured; the capillary is removed for alteration and the flow remeasured until the proper size has been found, after which the glass is sealed in place. Once fixed, there should be no need for change, and since the gas flows upward through the capillary, it cannot become clogged by foreign matter.

If desired, the device may be calibrated by measuring the flow under a few different pressure heads. The pressure may be varied by use of the bulb, *E*. The results of such a calibration are reproducible and, when plotted, may be interpolated to give an exact gage of the flow for any pressure. This is a convenience if the sweeping out process at the end of a combustion is to be hastened. If a single flow is sufficient, there is no need for *E*, and the overflow tube may be sealed off at the bottom and filled with the proper amount of mercury.

In operation, the oxygen is permitted to overflow in *B* at a rate of about one bubble a second. At this rate of overflow there is no appreciable variation in the pressure.

Semimicro- and Micro-Kjeldahl Steam-Distillation Unit

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THE steam-distillation of liberated ammonia in the Kjeldahl nitrogen determination has been the subject of considerable study.

The well-known apparatus of Parnas and Wagner, utilized by Pregl (*6*), is familiar to those concerned with microanalyses. Its chief difficulties lie in its bulkiness and the necessity for an elaborately constructed vacuum-insulated distilling chamber.

This latter fault has been eliminated in various ways. Kemmerer and Hallett (*4*) used an improved steam-generating unit, which was only a slight improvement over the original apparatus. Fife (*2*) placed a steam chamber between the steam generator and the distilling chamber. Allen (*1*) clamped his digestion flask to a steam generator. Redemann (*7*) modified Allen's design and adapted it to the semimicrodetermination. The one-piece, all-glass apparatus of Kirk (*5*) was a vast improvement in design. In place of a vacuum-insulated distilling chamber, he used a steam-jacketed chamber, the steam from which entered the distilling chamber and effected the distillation.

The apparatus described here is a modified form of the Kirk apparatus, simpler in construction and made more flexible by the use of standard taper joints.

The construction of the apparatus is apparent from Figure 1. The volume of the distilling chamber is large enough to permit vigorous distillation without frothing and mechanical movement of solution into bulb *A*.

Operation

The procedure follows the method given by Harte (*3*). A sample, sufficient to yield 2 to 5 mg. of nitrogen, is digested in a 100-cc. Kjeldahl flask with sulfuric acid and any of the well-known catalysts. When digestion is complete, the sample is cooled, diluted with 20 cc. of ammonia-free distilled water, and transferred to the distilling chamber, *A*, by means of the funnel, *B*. Both the Kjeldahl flask and the funnel are rinsed successively with three 5-cc. portions of water.

Stopcock *C* is closed and the condenser tube, *D*, is immersed in a known quantity of standard acid in a flask, *E* (not shown). Sodium hydroxide (50 per cent) is added to the sample in *A* through *B* and the funnel is again rinsed down with three 5-cc. portions of water. This is adequate to ensure complete removal of sample and caustic from the funnel and bulb, *A*, to the distilling chamber, *A*.

Although the apparatus has been found entirely satisfactory as described, a tip attached to funnel *B*, extending into bulb *A*, as discussed by Redemann (*7*), represents a possible modification.

The distilling tube, *A*, is now about one-third full. A 200-cc. round-bottomed flask, *F* (not shown), containing 100 cc. of ammonia-free distilled water, is connected to *G*. A flame is applied to *F* and the steam generated heats the jacket, *H*, preventing condensation in *A*, and enters the distilling chamber through tube *I*. This requires about 4 minutes' heating time. After allowing the distillation to proceed for 10 minutes, *E* is lowered and the distillation is continued for another 2 minutes to ensure complete rinsing of the condenser tube. The end of the condenser tube is then rinsed off with two 3-cc. portions of water and the flask is removed. The excess of acid is determined by titrating with standard alkali, using methyl red as the indicator. The flame is removed before the titration is made. As the generating flask, *F*, cools, the residue in *A* is withdrawn through *I*. Successive rinsings with distilled water, introduced at *B*, will be removed through *I* as *F* continues to cool.

Before using, the apparatus is steamed out thoroughly by distilling 100 cc. of water through it. As long as the flame is kept at *F* no sucking back is caused by contact of steam on tube *A* and walls of *G*.

Table I. Determination of Nitrogen
(Nitrogen present, 4.087 mg.)

Time of Distillation	Nitrogen Found	Error		Deviation from Mean	
Min.	*Mg.*	*Mg.*	*%*	*Mg.*	*%*
10	4.085	−0.002	−0.05	±0.000	±0.00
	4.082	−0.005	−0.12	−0.003	−0.07
	4.088	+0.001	+0.02	+0.003	+0.07
	4.082	−0.005	−0.12	−0.003	−0.07
	4.090	+0.003	+0.07	+0.005	+0.12
	4.080	−0.007	−0.17	−0.005	−0.12
4	4.080	−0.007	−0.17	−0.005	−0.12
	4.082	−0.005	−0.12	−0.003	−0.07
	4.088	+0.001	+0.02	+0.003	+0.07
	4.090	+0.003	+0.07	+0.005	+0.12
Av.	4.085				

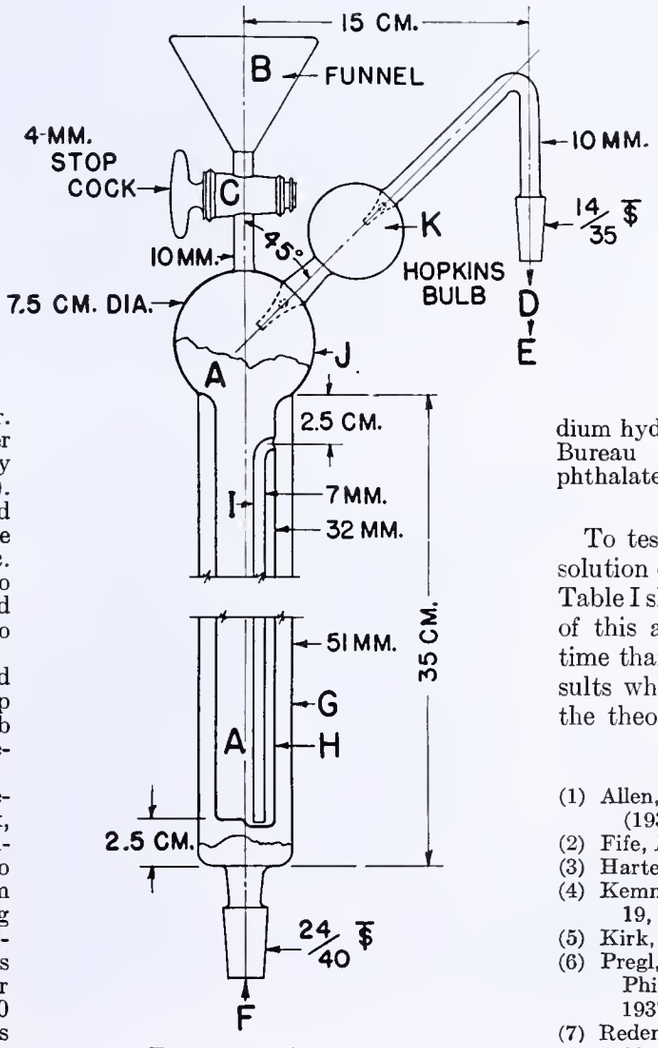

Figure 1. Apparatus

The complete outfit may be mounted on one ring stand and thus is completely portable. The total height of 95 cm. (38 inches), including the burner, is no great inconvenience and is compensated for by the simplicity of design and stability.

The water used was distilled off alkaline permanganate until it showed no ammonia by Nessler's reagent. The standard acid was prepared from constant-boiling hydrochloric acid. The standard alkali was prepared from 50 per cent sodium hydroxide and standardized against Bureau of Standards acid potassium phthalate.

Performance

To test the performance, a standard solution of ammonium sulfate was used. Table I shows the results obtained by use of this apparatus. Shorter periods of time than those recommended give results which are in good agreement with the theoretical figures.

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MODERN LABORATORIES

McGilvrey Hall at Kent State University

C. F. RUMOLD
Kent State University, Kent, Ohio

OF THE five state universities in Ohio, Kent State University is the unit for the northeastern section of the state. McGilvrey Hall is its new science building, constructed as a joint state and federal project begun late in 1939 and completed by November, 1940, at a cost of $1,153,386.

The building has four floors and is in the shape of an L. It is placed on the northerly slope of the glacial terminal moraine in such position that ground entrance is made to each of the first three floors. It is supported throughout by a heavy continuous steel skeleton entirely concealed within the masonry. The walls are of building brick and tile, faced on the outside with brownish-white glazed brick. The trimming is of native sandstone. The corridor walls have their lower two fifths faced with brownish glazed tile brick. The walls and ceilings are of acoustic plaster, and the floors are of heavy concrete base surfaced with composition slab tiles.

McGilvrey Hall has two wings, A and B. The ceiling-floor distance throughout each is 12 feet. Except in the end rooms, the room depth is 24 feet, and the corridors are 10 feet wide. The room partitions are of removable tile or light wood and plaster to admit of ready changes.

Wing A is 236 feet long and has end parts 74 feet wide and mid-bays 65 feet wide. It is used by the Chemistry and Physics Department. The easterly end is connected by a tunnel to Kent Hall to give access to all the main buildings of the university. Over the tunnel is a promenade giving open-air communication to the same buildings.

Wing B, 180 feet long and 65 feet wide, is used by the Departments of Biology and Geography. A connecting passage bridges over the ground floor at the north end and provides communication at each level between the second, third, and fourth floors. The ground space under the bridge affords access for vehicles from Lincoln Avenue to the loading platforms in the angle made by the back walls of the two wings.

All the plumbing, piping, and conduits are exposed and are carried in stirrups suspended 2 feet under the ceilings within the rooms. The heating and ventilating system is of the univent-in-outer-wall type, operated in conjunction with a

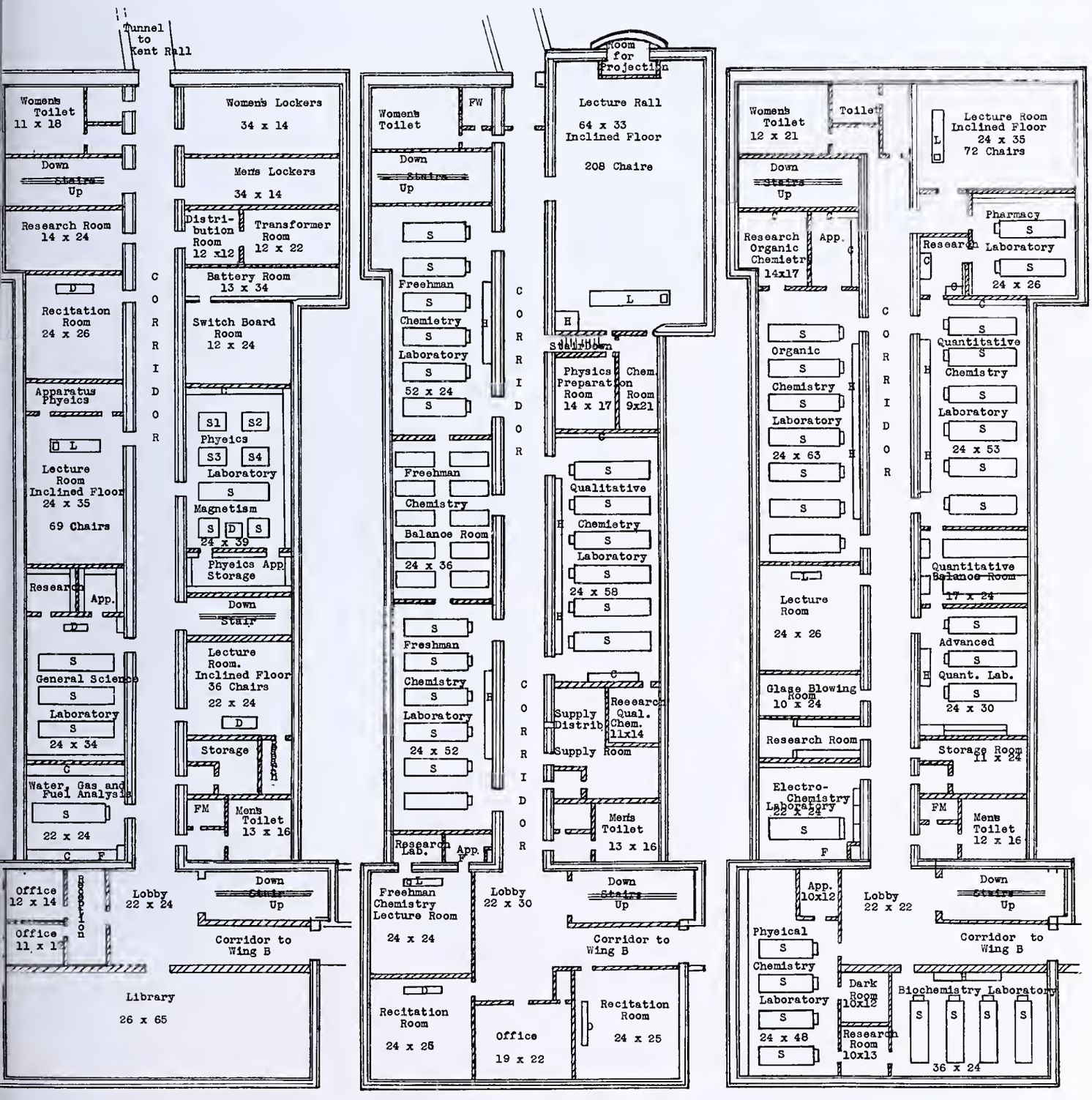
North Side View of Wing A, Used by Departments of Chemistry and Physics

system of exhaust and blower fans located in a penthouse on the roof. Heat is supplied by steam from the university's central power plant. The lighting is on a liberal scale and is provided by an indirect system of lamps in white globes, using alternating current at 115 volts.

ELECTRIC POWER. Both alternating and direct current is distributed to all parts of the building. The alternating current is provided by a set of transformers taking energy from the outside commercial high-tension line and delivering it at 220 and 115 volts to a main panel which distributes current to unit distributing panels. The main distribution panel is of the enclosed safety type which permits access to any separate circuit only after it has been disconnected from its current source. Each circuit in the system is protected by an automatic cutout.

High-tension alternating current may be drawn at the main distributing panel and is led to the electrochemistry laboratory and to the advanced physics laboratory.

The direct current is furnished by two storage batteries of the lead accumulator type charged by a motor generator set. One battery delivers a maximum voltage of 220 volts and the other a maximum voltage of 110 volts. Each battery delivers current to the same common distribution panel, from which any desired voltages can be delivered to unit panels in the different


Tunnel to Kent Hall
Women's Toilet 11 x 18
Women's Lockers 34 x 14
Men's Lockers 34 x 14
Down Stairs Up
Research Room 14 x 24
Distribution Room 12 x12
Transformer Room 12 x 22
Battery Room 13 x 34
Recitation Room 24 x 26
Switch Board Room 12 x 24
Apparatus Physeics
Lecture Room Inclined Floor 24 x 35 69 Chairs
Physeics S1 S2 S3 S4 Laboratory
Magnetism 24 x 39
Physeics App. Storage
Research App.
Down Stair
Lecture Room. Inclined Floor 36 Chairs 22 x 24
General Science Laboratory 24 x 34
Storage
Water, Gas and Fuel Analysis 22 x 24
FM Men's Toilet 13 x 16
Office 12 x 14
Office 11 x 14
Lobby 22 x 24
Down Stairs Up
Corridor to Wing B
Library 26 x 65
CORRIDOR
Women's Toilet
FW
Down Stairs Up
Freshman Chemistry Laboratory 52 x 24
Freshman Chemistry Laboratory 24 x 52
Balance Room 24 x 36
Freshman Chemistry Lecture Room 24 x 24
Research Lab. App.
Lobby 22 x 30
Room for Projection
Lecture Rall 64 x 33 Inclined Floor 208 Chaire
Physics Preparation Room 14 x 17
Chem Room 9x21
Qualitative Chemistry Laboratory 24 x 58
Supply Distrib
Research Qual. Chem. 11x14
Supply Room
Men's Toilet 13 x 16
Down Stairs Up
Corridor to Wing B
Recitation Room 24 x 25
Office 19 x 22
Recitation Room 24 x 25
Women's Toilet 12 x 21
Toilet
Lecture Room Inclined Floor 24 x 35 72 Chairs
Pharmacy
Research Organic Chemistry 14x17
App.
Research Laboratory 24 x 26
Organic Chemistry Laboratory 24 x 63
Quantitative Chemistry Laboratory 24 x 53
Lecture Room 24 x 26
Quantitative Balance Room 17 x 24
Advanced Quant. Lab. 24 x 30
Glass Blowing Room 10 x 24
Research Room
Storage Room 11 x 24
Electro-Chemistry Laboratory 22 x 22
FM Men's Toilet 12 x 16
App. 10x12
Lobby 22 x 22
Down Stairs Up
Corridor to Wing B
Physeical Chemistry Laboratory 24 x 48
Dark Room 10x12
Research Room 10x13
Biochemistry Laboratory 36 x 24

PLANS OF SECOND, THIRD, AND FOURTH FLOORS OF MCGILVREY HALL, WING A

C. Storage cupboard
D. Dumb-waiter
F. Focault pendulum shaft
FM. Faculty men's toilet
FW. Faculty women's toilet
H. Fume hood
L. Lecture table
S. Student work table

rooms, permitting wide ranges of voltages and currents to the terminals on desks and work tables.

Distilled Water. Distilled water is furnished by two completely automatic Barnstead stills, operated with either steam or gas, which have capacities of 5 gallons per hour for wing A and 2 gallons per hour for wing B. These stills are located in penthouses. The distilled water is distributed by gravity through aluminum pipes to one point in each laboratory.

Hydrogen Sulfide. Hydrogen sulfide is delivered from a gasometer in the penthouse on the roof of wing A to each hood compartment in the two freshman chemistry laboratories and in the qualitative chemistry laboratory through rubber-fabric pipes. The gasometer is charged from commercial cylinders.

Elevator. The elevator, located in wing A, is an Otis automatic electric type. Access to the carriage is had from the corridor and from the storage room on each floor.

General Facilities. Alternating and direct current, gas, air pressure at 15 pounds, vacuum, and hot and cold water are provided at each lecture table, student's work table, and hood, and at one or more places along the walls of each laboratory and lecture room. These facilities are liberal in each of the research rooms.

The compressed air pump and the vacuum pump are operated by electricity and are completely automatic. The vacuum pump is protected by a filter against solids and corrosive liquids and gases.

Wing A has in its central part a Focault pendulum shaft which permits of a suspension of 80 feet in the clear.

The striking features of the building are the liberality of the lighting, the large ventilating capacity, the large number of electrical outlets and of gas cocks, and the easy accessibility to hot and cold water, and each laboratory and recitation room has at least one commodious research room and an ample storage room attached to it and leading directly off from it.

Physics Laboratories

The freshman, second year, and advanced physics laboratories are located on the ground floor of wing A. Each student work table is equipped with 8 alternating current 115-volt outlets, 8 direct current outlets, 16 gas cocks, 8 vacuum cocks, 8 pressure cocks, and a central sink with 4 cold and 2 hot water faucets. Each laboratory has wall work tables equipped with the same number of each kind of service as the main tables.

The freshman physics laboratory and the advanced physics laboratory each has a darkroom attached and all three have large apparatus rooms attached. A fully equipped photography room is attached to the darkroom of the freshman physics laboratory.

The magnetism laboratory is on the second floor of wing A. It has four work desks of solid masonry without metal, 4 by 6 feet, with Alberene tops 4 inches thick. Electric current is available from an independent outlet rising from the floor alongside each table. These nonmagnetic tables are built up from the firm ground under the floor. Each student table in this laboratory is equipped with 2 alternating current 220-volt, 2 alternating current 115-volt, 2 direct current outlets, and 2 gas cocks.

General Science Laboratory

The general science laboratory is located on the second floor of wing A. The student work tables are equipped on each side with 6 alternating current 115-volt and 6 direct current outlets, 6 gas cocks, and a central sink with hot and cold water. A research laboratory and an apparatus storage room are attached.

Chemistry Laboratories

Each laboratory has liberal hood facilities along the inner walls and on the work tables. All hoods are provided with liberal connections for alternating and direct current, gas, hydrogen sulfide, distilled water, and hot and cold water, and with sinks between the hood compartments. One or two showers in each laboratory are conveniently located for use in case of accidental catching fire of clothing. The lighting in the laboratories is on an unusually liberal scale and is brilliant, one 200-watt 115-volt Mazda lamp per 83 square feet of floor surface, approximately throughout. All drawers and lockers have a master-keyed system of locks for use by the department when the places are not assigned to students; also hasps for student use with locks of their own purchase.

Each student table in the two freshman laboratories and in the qualitative chemistry laboratory has on each side 40 drawers for equipment, giving 960 work places for students in freshman chemistry and 480 in qualitative chemistry. These tables are further equipped on each side with 5 alternating current 115-volt and 5 direct current outlets, 5 pressure outlets, 5 cold water taps, 10 gas cocks, and 3 down-draft fume hoods.

A balance room accommodating 52 balances on 13 tables is located between the two freshman chemistry laboratories. The lighting here is brilliant, there being eight 200-watt Mazda lamps over the 840 square feet of floor surface.

In the organic, quantitative, physical chemistry, biochemistry, and pharmacy laboratories each student work table has on each side 9 drawers with a locker under each for student equipment, giving 126 places for students in organic chemistry, 162 places in quantitative analysis, 72 places in biochemistry, 36 places in pharmacy, and 72 places in physical chemistry.

Each work table in the organic, quantitative, physical, biochemistry, and pharmacy laboratories is supplied on each side with 3 alternating current 115-volt, 3 direct current outlets, 3 vacuum taps, 3 pressure taps, 3 cold water taps, 3 steam taps, 6 gas cocks, and 3 down-draft fume hoods.

An ample research room and a storage room are connected with each of these laboratories. The physical chemistry room has in addition a darkroom and a large direct current distributing panel.

The balance room between the two quantitative laboratories accommodates 24 balances on 6 tables. The lighting in this room is supplied by four 200-watt Mazda lamps.

Wing B

Wing B has laboratories and recitation rooms for physiology, comparative anatomy, ecology, general zoology, general botany, bacteriology, pathology, plant physiology, geology, and geography. Each laboratory has liberal space for side-wall cupboards, storage rooms, and research rooms. The comparative anatomy, ecology, general zoology, general botany, and general biology laboratories are equipped with student work tables designed to have work space on one side only, to permit all students to face the same direction for instructional purposes. Each work table is equipped with 24 drawers for student equipment and 6 alternating current 115-volt outlets. Hot and cold water are available at end sinks. Additional sinks and work-table spaces are provided along the walls.

In the physiology laboratory each student table is provided on each side with 12 lockers and 12 drawers, alternating current 115-volt and 3 direct current outlets, 12 gas cocks, and 12 pressure taps. Hot and cold water are available at the end sinks. This laboratory also has a large fume hood and a large direct current distribution panel.

Each student work table in the bacteriology laboratory has 24 drawers on each side, 8 gas cocks, 4 cold water taps, and 4 electrical outlets. Hot and cold water are available at the end sinks.

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Determination and Separation of Potassium as Periodate

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THE determination of potassium as periodate, KIO_4, offers a number of advantages. It has a high molecular weight, and can be determined volumetrically by a very exact titration. It is only sparingly soluble, a saturated solution at 25° C. being 0.022 molar (*2*). It contains about the same percentage of potassium as the chloroplatinate and cobaltinitrite and much less than the perchlorate.

The first experiments along this line were made by Greathouse (*1*), who added periodic acid to the solution, 2 or 3 ml. in volume, and completed the precipitation by adding alcohol, free from aldehyde. He carried out a few gravimetric and volumetric determinations, the results being usually slightly low, but did very little on the separation of potassium from other metals. At that time the price of iodine was high, and the preparation of periodic acid was an expensive process. Since that time two satisfactory methods for preparing the acid have been published and the reagent has become less expensive. As the reagent is now available, it seemed desirable to make an extensive investigation of this method of determining and separating potassium, and in particular, to find a better solvent than ethyl alcohol which is so easily oxidized that it is almost impossible to avoid some reduction of periodic acid to iodic acid.

The reaction used in titrating periodate to iodate was first suggested by Müller and Friedberger (*3*):

$$IO_4^- + 2I^- = IO_3^- + I_2$$

and is quantitative only in a neutral solution buffered by carbonic acid–bicarbonate or boric acid–borate, preferably the latter. The free iodine is then titrated by standard arsenite. This method has the advantage that iodate does not interfere, as it would if the periodate were reduced to iodide. In the latter process, however, the equivalent would be much smaller—one eighth of the molecular weight.

The periodate method for potassium is both rapid and accurate, and can be applied to amounts of potassium as low as 0.4 mg.

Experimental

SELECTION OF A SOLVENT. As indicated above, the selection of the proper solvent is a matter of prime importance. It must possess the following properties: (1) It must not be appreciably oxidized by periodic acid during the time required for the analysis, 0.5 to 2 hours. (2) It should be miscible with water at least to the extent of about 10 per cent of the latter by volume. (3) Potassium periodate must be insoluble in the solvent and yet it must dissolve sodium periodate and such other salts as might be present.

As explained above, Greathouse (*1*) used ethyl alcohol, free from aldehyde, since the aldehyde is more easily oxidized than the alcohol. This, however, was not an ideal solvent because some oxidation of the alcohol invariably occurred. Among the solvents which are less affected by oxidizing agents is tertiary butyl alcohol; this was therefore first investigated. It was found, however, to dissolve so little sodium periodate as to be useless for the purpose. Admixture of ethyl acetate did not improve matters. Other solvents tried in which sodium periodate was insoluble were dioxane, the carbitols, Cellosolve, and diethylene glycol.

It was found that if ethyl acetate was added to ethyl alcohol, the resistance of the latter to oxidation was considerably increased, whereas its solvent power was not diminished.

A quantity of 95 per cent ethyl alcohol, free from aldehyde, was prepared by refluxing the alcohol for 2 or 3 hours after the addition of 0.5 gram of sodium hydroxide and 2.5 grams of silver nitrate per liter. The alcohol was then distilled and mixed with an equal volume of anhydrous ethyl acetate. The mixture was miscible with the quantities of water which were required and showed considerable resistance to oxidation by periodic acid. One gram of periodic acid dissolved in 100 ml. of the solvent showed the first traces of free iodine after standing at room temperature for 24 hours. Since in most cases the time required for the separation of potassium was from 15 minutes to 1 hour, it is apparent that only very slight oxidation of the organic solvent could occur. The magnitude of this effect is shown by the fact that the average of a long series of gravimetric determinations (only part of which are recorded in this paper) showed an error of +0.02 mg., and the volumetric determinations −0.05 mg. This would indicate that the precipitates contained a trace of iodate owing to very slight reduction by the solvent, because the percentage of potassium in the iodate is only slightly less than in the periodate, whereas the iodate is not determined at all in the volumetric process. These results are much better than those obtained by Greathouse (*1*). It was also found possible to reduce considerably the concentration of periodic acid.

PREPARATION OF REAGENTS. The periodic acid used was recrystallized from concentrated nitric acid until free from iodic acid. Some of it was prepared by the electrolytic process (*5*) and although originally free from iodic acid, some batches were found to contain a good deal of the latter

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after standing for a month or two. This is probably due to the catalytic effect of traces of colloidal platinum derived from the electrodes. A solution of periodic acid to which considerable colloidal platinum is added evolves oxygen rapidly. The acid prepared by the chemical process (*4*) is always entirely stable. Small amounts of iodate are easily detected as follows:

One-half gram of the periodic acid is dissolved in 25 ml. of water, a few drops of 0.25 *M* silver nitrate are added, and the solution is warmed until the yellowish-brown silver periodate dissolves, which should occur readily at about 80° C. If iodic acid is present, a white flocculent precipitate of silver iodate will form. If the brown silver periodate is not completely dissolved by heating, a drop or two of nitric acid, free from nitrous acid, should be added.

The potassium and sodium periodates used were purified by recrystallization and tested for iodate by the method of Willard and Thompson (*6*). This is similar to the method suggested above, except that most of the periodate in the sample to be tested is first removed by the addition of an excess of potassium nitrate, after which the solution is acidified with nitric acid and tested for iodate by the addition of silver nitrate. In this way it is possible to detect 0.03 per cent of iodate in periodate.

The potassium nitrate used in this work had been carefully purified and dried at 105° to 110° C. for several hours. An attempt was made to fuse the salt at about 300° C., but the resulting material contained considerable nitrite.

Table I. Determination of Potassium as Periodate in Presence and Absence of Sodium

Wt. of K as Nitrate	Na Added as Nitrate	Wt. of KIO_4	Error in K Gravimetric	Error in K Volumetric
Gram	*Mg.*	*Gram*	*Mg.*	*Mg.*
0.0794	...	0.4676	+0.1	...
0.0490	...	0.2887	+0.1	0.0
0.0405	...	0.2391	+0.1	+0.1
0.0382[a]	...	0.2241	−0.1	−0.1
0.0307	...	0.1809	0.0	−0.1
0.0224	...	0.1313	−0.1	...
0.0030	...	0.0171	−0.1	−0.1
0.0600	68	0.3524	−0.1	0.0
0.0428	73	0.2518	0.0	0.0
0.0464	135	0.2746	+0.3	+0.3
0.0420	135	0.2517	+0.8	+0.6
0.0481[a]	140	0.2842	+0.2	+0.2
0.0407[a]	143	0.2406	+0.2	+0.1
0.0440[a]	190	0.2598	+0.2	−0.1
0.0335[a]	190	0.1980	+0.1	0.0
0.0017	54	0.0098	0.0	...
0.0013	54	0.0077	0.0	−0.2
0.0007	54	0.0041	0.0	0.0
		5 ml. of water used		
0.0008	...	0.0045	0.0	+0.2
0.0008	...	0.0043	−0.1	0.0
0.0008	27	0.0045	0.0	0.0
0.0008	27	0.0052	+0.1	+0.1
0.0004	27	0.0025	0.0	−0.1
0.0004	27	0.0020	−0.1	−0.1

[a] 180 ml. of alcohol-acetate mixture used.

General Procedure

A sample weighing 0.1 to 0.2 gram is dissolved in water in a 150-ml. beaker. If potassium chloride is used it must be evaporated to dryness with 10 ml. of concentrated nitric acid; otherwise the chloride will cause reduction of periodic acid. To the nitrate dissolved in 4 to 5 ml. of water 3 ml. of water containing 1 gram of periodic acid are added, the mixture is stirred, and 3 or 4 minutes are allowed for the potassium periodate to precipitate. Ninety milliliters of the alcohol–ethyl acetate mixture are added and the solution is allowed to stand in an ice bath for 0.5 hour with continuous mechanical stirring. It is essential that the precipitation be carried out as indicated. If periodic acid is added to the alcohol–ethyl acetate solution, the precipitate will be gelatinous and difficult to filter, whereas if it is first formed in aqueous solution it is crystalline. The solution is filtered through a Gooch crucible and the precipitate washed with anhydrous ethyl acetate which has been cooled to 0°. It is dried in the oven for 10 minutes at 105° C., cooled, and weighed.

If volumetric results are desired, the crucible with the precipitate is placed in a 250-ml. beaker, to which are added 125 ml. of a solution containing 5 grams of boric acid and 5 grams of sodium tetraborate. The potassium periodate dissolves readily in this solution, which has a pH of about 7.5. It is unnecessary to take the crucible out of the solution.

When the potassium periodate has dissolved, 3 grams of potassium iodide are added and the iodine is titrated with 0.1 *N* arsenite solution, prepared by dissolving 4.945 grams of arsenious oxide in a solution of 10 grams of sodium bicarbonate, warmed to about 80° C. The solution is then saturated with carbon dioxide and diluted to 1 liter. Although the solution was made up from arsenious oxide of known purity, it was standardized by titration against pure potassium and sodium periodates and the values obtained in this way were taken as correct. In some cases the normality found in this way deviated from the theoretical value by as much as 0.0012.

Periodic acid was always weighed out in the solid form and dissolved just before use to avoid any danger of decomposition on standing. Gravimetric results were satisfactory only when a nitrate was used, but were high with sulfates, though volumetrically the sulfates did not interfere. The latter procedure is usually preferable because it is not affected by the presence of iodate or of inert materials which may be insoluble in the organic solvent.

When the amount of potassium present is very small, it is desirable to increase the amount of periodic acid to 1.5 or 2 grams. If not much sodium or other metal is present, the initial volume of water should be decreased to 5 ml., although this is not absolutely necessary. The time for precipitation should be increased to 1 or 1.5 hours. If less than 0.4 mg. of potassium is present the results are unreliable, even though the volume of water is reduced and the amount of periodic acid increased.

Table II. Determination of Potassium as Periodate in Presence of Free Acid

Wt. of K as Nitrate	Acid Added	Wt. of KIO_4	Error in K Gravimetric	Error in K Volumetric
Gram		*Gram*	*Mg.*	*Mg.*
0.0524	0.5 ml. concd. HNO_3	0.3092	+0.2	0.0
0.0289	0.5 ml. concd. HNO_3	0.1695	−0.1	+0.1
0.0608	5 drops H_3PO_4, 85%	0.3575	0.0	−0.2
0.0561	5 drops H_3PO_4, 85%	0.3302	0.0	−0.1
0.0572	1 ml. concd. H_3PO_4	0.3377	+0.2	0.0
0.0603	1 ml. concd. H_3PO_4	0.3570	+0.4	+0.1
0.0461	5 drops concd. H_2SO_4	0.2806	+0.5	0.0
0.0580	5 drops concd. H_2SO_4	0.3408	−0.1	+0.1
0.0451	200 mg. H_3BO_3	0.2658	+0.1	0.0
0.0444	200 mg. H_3BO_3	0.2630	+0.3	+0.3
0.0447	5 drops $HClO_4$, 70%[a]	0.2623	−0.1	−0.4
0.0541	5 drops $HClO_4$, 70%[a]	0.3179	−0.1	−0.5

[a] Stirred 45 minutes.

Results of Analyses

In all the analyses described below, unless otherwise stated, the same conditions were used—namely, 90 ml. of solvent, 1 gram of periodic acid, 7 to 8 ml. of water, and 30 minutes stirring at 0° C. The precipitate obtained was, in all cases, crystalline, and showed no tendency to adhere to the beaker. The results of a series of determinations are shown in Table I.

It is obvious that potassium can be completely separated from 70 mg. of sodium, but that there is a slight error when 140 mg. are present. It was subsequently found that the limit could be raised to 100 mg. By doubling the volume of alcohol-acetate mixture potassium was readily separated from 190 mg. of sodium.

Effect of Temperature, Sulfate, and Free Acid

In previous experiments the temperature was 0° C. A series of experiments was run in which the solutions were maintained at room temperature for 15 and 60 minutes, respectively. In both cases the results were 0.2 to 0.6 mg. too low.

Weights of potassium nitrate varying from 0.05 to 0.15 gram were taken and 40 mg. of sodium added as sulfate. In a series of 12 volumetric determinations the error varied from

−0.2 to +0.2 mg., with an average of practically zero. In this case the gravimetric results were always too high because of contamination with sodium sulfate.

In Table II are shown the results obtained when free nitric, phosphoric, sulfuric, perchloric, and boric acids are added. In the case of perchloric acid, some of the potassium is precipitated as perchlorate and apparently is not quite converted into periodate within 45 minutes. The other acids do not interfere.

Table III. Separation of Potassium from Metals as Periodate

Wt. of K as Nitrate	Metal Added	Wt. of KIO_4	Error in K Gravimetric	Error in K Volumetric
Gram	*Mg.*	*Gram*	*Mg.*	*Mg.*
0.0403	16 Ca (as nitrate)	0.2731	0.0	−0.1
0.0530	32 Ca (as nitrate)	0.3119	0.0	−0.1
0.0486	20 Mg (as sulfate)	....	...	0.0
0.0464	21 Mg (as nitrate)	0.2732	+0.1	0.0
0.0608	50 Al (as nitrate)	0.3579	0.0	−0.1
0.0615	100 Al (as nitrate)	0.3654	+1.1	−0.1
0.0452	102 Al (as nitrate)	0.2718	+1.0	0.0
0.0405[a]	98 Al (as nitrate)	0.2468	+0.5	+0.1
0.0424	100 Al (as sulfate)	0.2530	+0.6	0.0
0.0400	100 Al (as sulfate)	0.2383	+0.3	+0.2
0.0484	62 Zn (as nitrate)	0.2876	+0.5	−0.1
0.0406	62 Zn (as nitrate)	....	...	−0.1
0.0498	92 Co (as nitrate)	0.2959	+0.5	−0.2
0.0406	88 Co (as nitrate)	0.2400	+0.2	+0.2
0.0423	77 Ni (as nitrate)	0.2513	+0.4	+0.2
0.0395[a]	79 Ni (as nitrate)	0.2336	+0.2	+0.2
0.0413	5 Fe^{+++} (as nitrate)	0.2773	+5.8	−0.9
0.0358	5 Fe^{+++} + 5 drops H_3PO_4	0.2355	+4.2	−1.3
0.0579	50 Mn (as sulfate)	0.3757	+6.0	...
0.0341	3 NH_4 (as nitrate)	0.2243	+4.0	+4.6
0.0516	60 Li (as carbonate)	0.3037	0.0	...
0.0401	69 Li (as carbonate)	0.2371	+0.2	−0.1

[a] 180 ml. alcohol-acetate mixture used.

Separation from Other Metals

The results in Table III show that volumetric determination of potassium is satisfactory in the presence of moderate amounts of magnesium, calcium, lithium, aluminum, zinc, nickel, and cobalt, but not ammonium, iron, manganese, and chromium. In the latter cases oxidation to permanganate and chromate occurs and the titration is not possible. The separations are better when 180 ml. of alcohol-acetate mixture are used. The results are somewhat better when 8 ml. of water rather than 7 ml. are present.

The gravimetric results are satisfactory with calcium (in the absence of sulfate), magnesium, and lithium.

Although calcium does not interfere when present as nitrate, there is serious interference when it is present as sulfate. Not only was the reaction at the end point slow, indicating that something was dissolving during the process, but the results were always too low. Apparently this is due to the formation of the insoluble double salt, $CaSO_4.K_2SO_4$, so that it was impossible to convert all the potassium to periodate. This should be kept in mind if silicates have been decomposed by evaporation with hydrofluoric and sulfuric acids. Attempts to decompose feldspar by evaporation with hydrofluoric and phosphoric acids were unsuccessful.

The cobalt solution became green upon the addition of periodic acid. Ferric iron is readily precipitated as periodate and therefore interferes even when present in small amounts. It was thought that the addition of phosphoric acid, forming a complex with the iron, would prevent its precipitation. Although some effect was noticeable in the gravimetric result, there was no improvement in the volumetric. In the absence of phosphoric acid the precipitate was brick-red, whereas in its presence it was white.

Rubidium and cesium behave like potassium, although no quantitative experiments were made.

In the presence of chromium and manganese, which are oxidized by periodic acid, the color was so intense that no titration was possible, although if not over 1 mg. of manganese is present the end point can be seen.

The periodate method is applicable to the determination of potassium in the mixed chlorides obtained by the J. Lawrence Smith method, providing they are converted into nitrates by evaporation with nitric acid.

Summary

Potassium can be quantitatively precipitated as periodate by adding periodic acid to a solution only a few milliliters in volume and subsequently completing the precipitation by the addition of a much larger volume of a mixture of equal parts of aldehyde-free ethyl alcohol and anhydrous ethyl acetate.

The solution must be maintained at 0° C. and stirred for 30 minutes.

The precipitate of potassium periodate may be weighed or it may be dissolved in a boric acid–borax buffer, potassium iodide added, and the free iodine titrated with arsenite. In this reaction the periodate is reduced to iodate.

Potassium may be separated from calcium, magnesium, zinc, aluminum, sodium, lithium, nickel, and cobalt, but not from manganese, iron, chromium, rubidium, cesium, and ammonium. It is possible to separate as little as 0.4 mg. of potassium from seventy times as much sodium. If both calcium and sulfate are present the results are too low, probably because of the formation of a double potassium calcium sulfate.

Small amounts of sulfuric, phosphoric, nitric, and boric acids may be present, but with large amounts the precipitate becomes gelatinous and difficult to filter. In the presence of sulfate the gravimetric determination is impossible, but the volumetric method gives satisfactory results. Chloride must be absent.

The method is rapid and accurate.

Acknowledgment

The authors wish to express their thanks to Robert McNulty, who performed some of the analyses in Table III.

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From a thesis presented by Albert J. Boyle to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of doctor of philosophy.

Correction. The following corrections should be made to Figure 1 of our article, "An Electronic Relay" [Ind. Eng. Chem., Anal. Ed., 12, 757 (1940)]:

1. In the relay circuit of Figure 1, interchange R_1' and R_1''.
2. In the diagram of the tube socket for the OA4G tube, move the cathode (small circle) connection one pin towards the right.

We wish to thank Professors Waddle and Serfass and Dr. Shomate for calling our attention to these errors.

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Recovery of Asphalts and Liquid Asphaltic Road Materials from Solution

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In evaluating the service behavior of paving asphalts, test samples must be retrieved from admixtures with mineral aggregates. Usually the binder can be extracted with a solvent and the solvent subsequently removed by some method of distillation. In this paper a method of fractionation and carbon dioxide sweeping is described for the separation of carbon disulfide or benzene from penetration asphalts. The method requires a somewhat more complicated apparatus than the Abson method, which is in wide use, but the results are more reliable, especially for cracked asphalts, which may contain light oils, or for asphalts of unknown origin. The apparatus is almost identical with one used by Siegmann for the recovery of liquid road materials. It may be used not only for penetration asphalts but also for liquid asphalts following Siegmann's procedure.

IN EVALUATING the service behavior of paving asphalts, test samples must be retrieved from admixtures with mineral aggregates, and it is important that the properties of the asphalt be changed as little as possible in this process.

Usually the binder can be extracted with a solvent, and the solvent subsequently removed by some method of distillation. Extraction techniques are described by Siegmann (*8*) and Chalk (*2*) for carbon disulfide, by Suida and Hoffmann (*9*) for benzene, and by Kamptner (*4*) for a variety of solvents. A centrifugal extractor (*10*) is approved by the American Association of State Highway Officials. This paper discusses subsequent separation of asphalt and solvent by distillation.

Table I. Abson Recoveries on West Texas Straight-Run and Cracked Asphalts

	Original Properties	Properties after Recovery by Abson's Method No. 1	No. 2
West Texas straight-run asphalt			
Change in weight[a] (75-gram sample), gram	..	+0.02	0.00
Penetration at 25° C.	99	99	98
Ring and ball softening point, ° C.	43.9	44.4	44.4
Penetration index (*5*)	−1.2	−1.0	−1.0
Ductility at 4° C. (5 cm. per min.)	6.6	6.7	7.0
Ductility at 25° C. (5 cm. per min.)	100+	100+	100+
West Texas cracked asphalt			
Change in weight (75-gram sample), gram	..	−0.04	−0.04
Penetration at 25° C.	105	98	98
Ring and ball softening point, ° C.	42.8	43.1	43.3
Penetration index	−1.4	−1.5	−1.4
Ductility at 4° C. (5 cm. per min.)	6.4	6.4	6.4
Ductility at 25° C. (5 cm. per min.)	100+	100+	100+

[a] Weighings accurate to ±0.01 gram.

Table II. Properties of West Texas Cracked Asphalts Recovered from Carbon Disulfide and Benzene

(Using modified Siegmann apparatus)

	Recovered from Carbon Disulfide		Recovered from Benzene	
	Original	Recovered	Original	Recovered
Penetration at 25° C.				
Sample 1	107	105	107	98
Sample 2	107	104	107	99
Sample 3	107	105	107	98
Ring and ball softening point, ° C.				
Sample 1	42.5	42.5	42.5	43.6
Sample 2	42.5	42.5	42.5	43.1
Sample 3	42.5	42.5	42.5	43.1
Weight of sample, grams[a]				
Sample 1	80.3	80.4	83.9	84.0
Sample 2	71.2	71.4	70.7	70.7
Sample 3	79.8	80.0	73.9	74.0

[a] Weighings accurate to ±0.2 gram.

Table III. Hardening Effect of Carbon Disulfide and Benzene on West Texas Cracked Asphalt

(Using modified Siegmann apparatus)

		Dissolved in Carbon Disulfide		Dissolved in Benzene	
	Original	Recovered immediately	Recovered after standing 22 hours in atmosphere of inert gas	Recovered immediately	Recovered after standing 22 hours in atmosphere of inert gas
Penetration at 25° C.					
Sample 1	107	105	98	99	98
Sample 2	107	105	98	100	98
Ring and ball softening point, ° C.					
Sample 1	42.5	42.5	43.9	43.3	43.1
Sample 2	42.5	42.5	43.6	43.6	43.6
Yield[a], weight %					
Sample 1	...	100.0	100.2	100.0	100.2
Sample 2	...	100.0	100.2	100.0	100.2
Sulfur, weight %					
Sample 1	3.41	3.40	3.39	[b]	[b]

[a] Yields accurate to ±0.3%.
[b] Not determined.

Distillation Methods

A method of fractionation and carbon dioxide sweeping is here described for the separation of carbon disulfide or benzene from penetration asphalts. It requires a somewhat more complicated apparatus than the widely used Abson method (*1*) but the results are more reliable, especially for cracked asphalts which may contain light oils or for asphalts of unknown origin. The apparatus may also be used for the recovery of liquid asphalts.

In Abson's method the solution of asphalt, in benzene, is distilled in a plain balloon flask at atmospheric pressure, and carbon dioxide is used to sweep out the last traces of solvent. The figures of Table I show, however, that with this method in some cases, as with cracked asphalts, asphaltic oils may also be swept out. Often the recovered distillates are colored. Such cases require a greater amount of fractionation than Abson's apparatus provides. The authors have therefore adopted for all asphalts a simple reflux column and flask used by Siegmann (*8*) for the recovery of liquid road materials from carbon disulfide by vacuum distillation, and have adapted it to the recovery of penetration asphalts by adding a capillary inlet to the flask to permit gas sweeping. They have found that gas sweeping is necessary with penetration asphalts to assure removal of the last traces of solvent. With gas sweeping, temperatures as low as 150° C. can be used. Vacuum at lower temperatures offers no advantage, since lower temperatures are usually attended by greatly increased viscosities which make mechanical agitation difficult.

Modified Siegmann Recovery

Procedure for Penetration Asphalts. The modified Siegmann apparatus adopted for the recovery of penetration asphalts is shown in Figure 1. The capacity of the flask is 1 liter, the capillary inlet is 1 mm. in inside diameter and reaches to within

TABLE IV. PROPERTIES OF ASPHALTS BEFORE AND AFTER RECOVERY FROM CARBON DISULFIDE SOLUTIONS

(Using modified Siegmann apparatus)

Source of Crude	Penetration at 25° C. Original	Penetration at 25° C. Recovered	Ring and Ball Softening Point Original ° C.	Ring and Ball Softening Point Recovered ° C.	Penetration Index Original	Penetration Index Recovered	Yield[a] Weight %
Straight-Run Asphalts							
California	22	22	55.6	55.6	−1.5	−1.5	99.7
	63	63	47.5	47.5	−1.3	−1.3	100.2
	97	97	43.9	43.9	−1.2	−1.2	100.0
	133	132	41.4	41.1	−1.2	−1.2	100.0
	231	226	36.4	36.4	−1.1	−1.2	99.8
Mexico	31	32	62.8	63.1	+0.5	+0.6	100.0
	57	56	55.8	55.8	+0.5	+0.5	100.0
	102	104	48.3	48.6	+0.4	+0.4	100.1
	159	156	43.6	43.6	+0.4	+0.4	100.0
West Texas	122	122	42.5	42.5	−1.0	−1.0	100.2
	99	98	43.9	44.7	−1.1	−0.9	100.1
Cracked Asphalts							
West Texas	35	33	52.8	54.2	−1.3	−1.1	100.0
	107	105	42.5	42.5	−1.4	−1.5	100.1
California	32	32	51.1	51.1	−1.8	−1.8	100.3
	108	105	42.2	42.5	−1.5	−1.5	100.0
Blown Asphalts, Final Temperature of Recovery 150° C.							
California	15.1	15.7	112.2	115.0	+5.1	+5.4	101.3
Mexico	24	29	94.4	93.3	+4.4	+4.7	102.1
	95	104	63.1	63.3	+3.6	+3.9	101.5
Blown Asphalts, Final Temperature of Recovery 200° C.							
California	15	15	112.2	111.4	+5.1	+5.0	99.5
Mexico	24	23.6	94.4	96.1	+4.4	+4.5	99.7

[a] Yields accurate to 0.3%.

3 mm. of the bottom of the flask, and the fractionating column is 25 cm. long and 8 mm. in inside diameter.

The asphalt solution, which as extracted usually contains 5 to 10 per cent of asphalt in carbon disulfide or benzene, is concentrated without vacuum in a flask provided with a reflux column to a concentration of approximately 25 per cent. About 400 cc. of this solution are introduced into the apparatus (Figure 1), a few silicon carbide boiling chips are added, and the solution is concentrated without vacuum or agitation over a water bath at 55° to 60° C. if carbon disulfide is the solvent; at 90° to 100° C. if benzene is the solvent. The bulk of the solvent is distilled under these conditions, the temperature being adjusted to maintain a distillation rate just short of a steady stream. When the temperature has reached 100° C. a previously heated oil bath is substituted for the water bath. The oil bath is then gradually heated to 150° C. when using either benzene or carbon disulfide and held at this temperature throughout the remainder of the recovery process.

After the distillation rate has dropped off to 5 to 10 drops per minute a stream of carbon dioxide gas is passed through the asphalt mass for 30 minutes at the rate of 900 cc. per minute. If at the end of this time condensed oils are visible in the fractionating column, careful heating will cause them to flow back into the flask. The flask is finally rotated rapidly to remix any oils condensed on its upper surfaces, and the recovered asphalt is ready for analysis.

As a safety precaution a filter flask filled with solid carbon dioxide should be attached to the open end of the apparatus. This will prevent air being accidentally drawn back into the carbon disulfide or benzene system.

TABLE V. COMPARISON OF ABSON AND MODIFIED SIEGMANN METHODS

(Approximately 75 grams of asphalt and 300 cc. of solvent were used throughout these experiments.)

	Original Properties	Properties after Recovery from Benzene by Abson's Method: Recoveries at 150° C. Maximum Temperature No. 1	No. 2	No. 3	No. 4	Recoveries at 163° C. Maximum Temperature No. 1	No. 2	Properties after Recovery from Carbon Disulfide in Modified Siegmann Apparatus No. 1	No. 2
West Texas straight-run asphalt:									
Yield, weight %[a]	..	.....	.....	.....	.....	100.0	100.0	100.0	100.1
Penetration at 25° C.	99	.....	.....	.....	.....	99	98	100	98
Ring and ball softening point, ° C.	43.9	.....	.....	.....	.....	44.4	44.4	44.7	44.7
Penetration index	−1.2	.....	.....	.....	.....	−1.0	−1.0	−0.8	−1.0
Ductility at 4° C. (5 cm. per min.)[b]	6.6	.....	.....	.....	.....	6.7	7.0	6.4	7.5
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	7.6	.....	.....	.....	.....	7.9	7.7	7.6	7.7
California straight-run asphalt:									
Yield, weight %	..	100.1	100.3	100.0	100.0	100.1	100.1	100.0	100.1
Penetration at 25° C.	97	103	103	97	109	102	95	97	97
Ring and ball softening point, ° C.	43.6	43.3	43.3	43.9	42.2	43.1	43.9	43.9	43.9
Penetration index	−1.3	−1.2	−1.2	−1.2	−1.5	−1.4	−1.3	−1.2	−1.2
Ductility at 4° C. (5 cm. per min.)	7.5	12.0	11.0	9.0	11.0	12.3	9.4	8.5	8.0
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	6.4	6.5	6.6	7.2	6.8	6.6	6.8	6.9	6.6
Mexican straight-run asphalt:									
Yield, weight %	..	100.1	100.0	100.0	.....	99.9	100.0	100.0	100.0
Penetration at 25° C.	103	107	100	105	.....	95	102	100	100
Ring and ball softening point, ° C.	48.3	48.3	48.9	48.3	.....	49.4	48.9	48.9	48.9
Penetration index	+0.3	+0.4	+0.4	+0.4	.....	+0.4	+0.4	+0.4	+0.4
Ductility at 4° C. (5 cm. per min.)	11.5	15.2	14.5	14.8	.....	10.2	13.0	11.5	12.9
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	19.7	20.8	21.3	20.8	.....	20.4	19.8	19.5	19.8
West Texas cracked asphalt:									
Yield, weight %	..	.....	.....	.....	.....	99.9	100.0	100.0	100.0
Penetration at 25° C.	105	.....	.....	.....	.....	98	98	102	101
Ring and ball softening point, ° C.	42.8	.....	.....	.....	.....	43.1	43.3	42.8	43.1
Penetration index	−1.4	.....	.....	.....	.....	−1.5	−1.4	−1.5	−1.4
Ductility at 4° C. (5 cm. per min.)	6.4	.....	.....	.....	.....	6.4	6.4	6.7	6.8
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	18.2	.....	.....	.....	.....	..	17.9	17.9	17.8
California cracked asphalt:									
Yield, weight %	..	100.1	100.3	100.0	100.0	99.9	99.9	100.0	100.0
Penetration at 25° C.	108	105	113	106	106	99	100	103	105
Ring and ball softening point, ° C.	42.2	42.8	41.7	42.0	42.8	42.5	42.8	42.5	42.5
Penetration index	−1.5	−1.4	−1.6	−1.6	−1.4	−1.7	−1.5	−1.6	−1.5
Ductility at 4° C. (5 cm. per min.)	8.4	..[c]	13.0	8.5	13.2	9.1	8.1	..[c]	8.4
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	15.2	15.1	15.4	15.9	15.4	15.2	15.3	15.5	15.3

[a] Yields accurate to 0.3%.
[b] All ductilities at 25° C. were above 100+.
[c] Low temperature ductilities broke at the start for 6 determinations.

TABLE VI. SUMMARY OF SIEGMANN RECOVERIES OF CUT-

Liquid Road Materials Designation	Mixture: Total weight Grams	Mixture: Yield %	Mixture: Viscosity Saybolt Furol At 25° C.	Mixture: Viscosity Saybolt Furol At 50° C.	Mixture: Viscosity Saybolt Furol At 60° C.	Asphalt: Source (all straight-run)	Asphalt: Total weight Grams	Asphalt: Penetration at 25° C.	Asphalt: Ring and ball softening point ° C.	Asphalt: Penetration index	Asphalt: Oliensis test
MC-1 cutback											
Original properties	279.6	...	104	...	..	California	141.4	115	42.8	−1.1	Neg.
Properties after recovery	280.0	100.1	103	...	..		141.8	105	43.3	−1.2	Neg.
MC-4 cutback											
Original properties	366.6	...	...	...	652	California	274.2	115	42.8	−1.1	Neg.
Properties after recovery	367.1	100.1	...	...	649		275.3	105	42.8	−1.4	Neg.
RC-1 cutback											
Original properties	333.7	...	...	94	..	California	212.2	103	45.3	−0.6	Neg.
Properties after recovery	330.5	99.1	...	105	..		214.2	90	45.0	−1.1	Neg.
RC-4 cutback											
Original properties	414.3	...	...	...	951	California	310.0	103	45.3	−0.6	Neg.
Properties after recovery	414.9	100.1	...	...	966		312.0	89	45.0	−1.1	Neg.
RC-1 cutback											
Original properties	332.8	...	...	80	..	Mexico	195.0	86	51.1	+0.5	Neg.
Properties after recovery	325.9	97.9	...	103	..		196.6	82	51.7	+0.6	Neg.
MC-4 cutback											
Original properties	346.6	...	...	...	487	Mexico	246.3	188	40.6	−0.1	Neg.
Properties after recovery	347.1	100.1	...	...	475		249.1	196	40.6	+0.1	Neg.
MC-1 cutback											
Original properties	285.0	...	94.6	...	..	California	149.0	94	44.2	−1.2	Neg.
Properties after recovery	286.3	100.4	88.1	...	..		149.2	96	43.9	−1.1	Neg.
RC-4 cutback											
Original properties	480.2	...	...	...	951	California	358.3	103	45.3	−0.6	Neg.
Properties after recovery	477.9	99.5	...	...	1086		359.7	87	45.0	−1.2	Neg.
SC-1 road oil											
Original properties	274.9	...	47.6	...	..						
Properties after recovery	276.0	100.4	40.3	...	..						
SC-2 road oil											
Original properties	287.6	...	...	285	..						
Properties after recovery	287.6	100.0	...	264	..						
SC-3 road oil											
Original properties	293.8	...	...	303	..						
Properties after recovery	294.5	100.2	...	261	..						
SC-4 road oil											
Original properties	294.9	...	...	...	453						
Properties after recovery	295.6	100.2	...	...	395						

In recovering 100-penetration low flash point cracked asphalt from benzene by this method no differences in yields or properties of the recovered asphalts were obtained by increasing the final bath temperature from 150° to 180° C., by increasing the time of passage of carbon dioxide from 30 to 80 minutes at 900 cc. per minute, or by varying the distillation rate between 100 and 200 drops per minute. Reducing the carbon dioxide rate from 900 to 450 cc. per minute resulted, however, in high yields and in softer recovered asphalts. In recoveries of the same asphalt from carbon disulfide, increasing the final bath temperature from 150° to 180° C. or increasing the time of passage of carbon dioxide from 30 to 80 minutes had no effect.

Data for parallel recoveries from carbon disulfide and from benzene are given in Table II. They show less hardening from carbon disulfide. These recoveries were made immediately after preparing the solutions, but as the solutions become older the differences tend to disappear. This behavior is illustrated by the figures of Table III. The authors have chosen to use carbon disulfide and recover as soon as possible. In many laboratories benzene may be preferred because of the great fire hazard with carbon disulfide. The benzene used may be the C. P. grade, or a commercial grade having a narrow distillation range and containing a negligible amount of high-boiling residue as determined by evaporation in a glass dish on the steam bath. Many commercial grades contain appreciable amounts of residue which will remain in the recovered asphalt and vitiate the results.

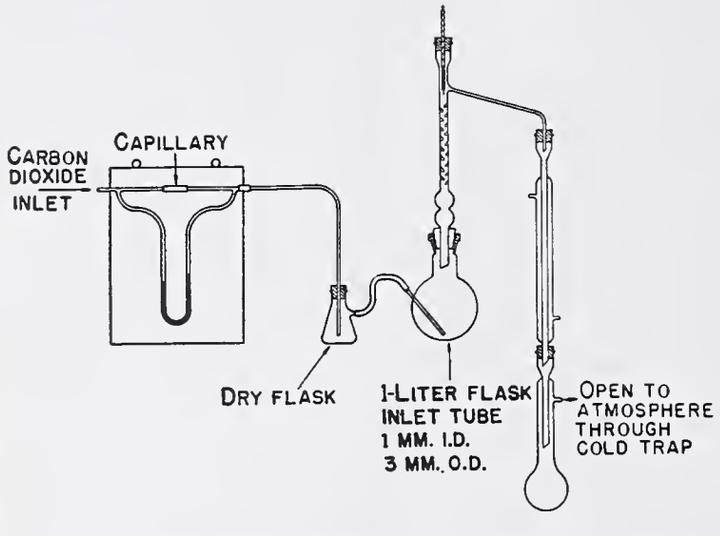

FIGURE 1. MODIFIED SIEGMANN RECOVERY APPARATUS

Greutert (*3*) and Pfrengle (*6*) report hardening in carbon disulfide which is greater if water is present, and Preston and Brandon (*7*) also give data on this point which appear conclusive. Thus, prior to extraction of paving samples, thorough drying is essential. The authors used dry asphalts and Baker's C. P. carbon disulfide in all recoveries here reported.

Chlorinated solvents as a class are considered reactive by most authors. Kamptner (*4*) states that in general chlorinated solvents make asphalts brittle. Solvents tested by him in the order of decreasing hardening effect are: carbon tetrachloride, trichloroethylene, dichloroethylene, chloroform, benzene, and carbon disulfide. Pfrengle (*6*) uses chloroform for recoveries and admits a hardening effect but attempts to apply a correction factor. Suida and Hoffmann (*9*) state that chloroform, carbon tetrachloride, and carbon disulfide but not benzene are reactive in the absence of air. Abson (*1*) on the other hand reports progressive hardening in benzene. In parallel recoveries on fresh solutions of 100-penetration asphalts the authors obtained: from chloroform, a drop in penetration of 22 points; from benzene, 9 points; and from carbon disulfide, 3 points.

Recoveries of Representative Penetration Asphalts

Results for recoveries of 18 straight-run, cracked, and blown asphalts from carbon disulfide by the procedure outlined above are given in Table IV. Of these 18 asphalts only the highly blown asphalts were too viscous at 150° C. to permit complete escape of the solvent. Better results were obtained at 200° C.

Results of parallel Siegmann and Abson recoveries of various grades of penetration asphalts are given in Table V. The recoveries by the Abson method are from benzene; by the authors method, from carbon disulfide.

Abson recommends a final temperature of 150° C. for 100-penetration asphalts and suggests 163° C. for asphalts of

BACKS AND ROAD OILS FROM CARBON DISULFIDE SOLUTION

Nature of fluxing agent	Total weight	Specific gravity 25°/4° C.	Fluxing Agent Used	Distillation (A. S. T. M. Apparatus) D86-35 Initial b. p.	5% over	10% over	20% over	30% over	40% over	50% over	60% over	70% over	80% over	90% over	95% over	Dry point	% over	% residue	Distn. loss
	Grams		*Cc.*	° *C.*															
Kerosene distillate	95.4	0.8377	100	200	204	207	209	212	214	217	220	224	229	236	245	251	97.7	2.2	0.1
	94.1	0.8371	100	191	203	207	209	212	214	217	220	223	228	235	242	258	98.5	1.3	0.2
Kerosene distillate	46.5	0.8377	50	198	203	206	209	212	214	217	221	224	230	238	248	257	97.2	2.6	0.2
	45.3	0.8397	50	195	202	207	210	213	216	218	222	226	231	240	...	257	96.0	3.0	1.0
Naphtha	75.0	0.7975	50	121	134	142	152	161	169	176	183	191	200	214	225	238	98.0	1.7	0.3
	73.3	0.8050	50	120	143	150	159	165	172	178	184	192	200	213	222	234	97.0	2.7	0.3
Naphtha	39.9	0.7975	50	121	134	142	152	161	169	176	183	191	200	214	225	238	98.0	1.7	0.3
	39.3	0.8196	40	64	106	157	162	168	174	180	186	190	203	220	236	238	95.5	3.4	1.1
Naphtha	85.5	0.7720	100	113	125	130	135	141	146	153	160	166	174	186	196	209	98.3	1.2	0.5
	84.1	0.7788	100	116	131	136	141	146	151	156	162	168	178	188	200	216	98.0	1.4	0.6
Cutback cracked gas oil	53.7	0.8312	50	170	180	187	195	200	205	211	218	224	234	245	257	264	96.8	2.7	0.5
	50.8	0.8331	50	168	182	189	195	200	205	212	215	222	229	242	254	264	96.2	2.9	0.9
Kerosene distillate	101.0	0.8355	100	198	203	206	208	211	213	215	218	221	226	234	241	250	98.5	1.1	0.4
	101.2	0.8371	100	181	203	206	208	210	212	214	218	221	225	233	239	246	98.4	1.3	0.3
Naphtha	44.2	0.7975	50	121	134	142	152	161	169	176	183	191	200	214	225	238	98.0	1.7	0.3
	43.4	0.8051	50	129	147	153	159	166	173	180	187	196	202	218	232	240	96.8	2.8	0.4

unknown penetration. Therefore, in Table V data are given for recoveries at both temperatures. At the higher temperature there is a tendency for asphalt oils to be lost, which results in hardening, and at the lower temperature solvent is sometimes retained, which causes softening. The authors have found that retained solvent is shown best by increased low-temperature ductility, and consequently, in those cases where the ductilities of the recovered asphalts are high and yields still not over 100 per cent, it must be concluded that losses of oils are compensated by retained solvent. The discrepancies when using the Abson method are not large and in many cases use of the somewhat more complicated Siegmann apparatus may not be justified. Nevertheless, whenever road oils or cracked asphalts may be encountered, the assurance of fractionation between asphalt oils and solvent seems necessary.

Recoveries of Liquid Road Materials by Siegmann's Method

The apparatus used here (Figure 1) for the recovery of penetration asphalts is Siegmann's apparatus for the recovery of liquid road materials, with the exception of the capillary inlet in the flask.

SIEGMANN'S PROCEDURE (*8*). The concentrated carbon disulfide extract is introduced into the flask of the Siegmann apparatus. The flask is placed in an oil bath, the temperature of which is raised carefully, so that the carbon disulfide passes over slowly (about 20 cc. per 15 minutes). When the greater part of the carbon disulfide has passed over, the apparatus is carefully subjected to vacuum, which is gradually increased to 40 cm. After the bulk of the carbon disulfide has been removed, the temperature of the oil bath is raised in 1 hour to 200° C.; upon continued heating the level of the fluxing agent is seen to rise slowly in the column. As soon as the vapors reach the bottom of the side tube of the fractionating column the vacuum is decreased, to prevent the fluxing agent from distilling over.

In the case of thinly liquid cutbacks, heating is continued until the thermometer reads 60° C., where the distillation is stopped.

With viscous cutbacks the thermometer reads 60° C. before the level of the fluxing agent vapors (visible by mist formation) has risen to the side tube. In such cases heating is then continued until the level of the side tube has been reached, which may take place at a temperature of about 85° C.

With kerosene cutbacks the oil bath has to be heated to at most 250° C., with creosote cutbacks to 300° C. to recover the cutbacks. If cutbacks that have lost nearly all their fluxing agent are to be examined, the oil bath will be at a temperature of 300° C. before the vapors reach the side tube. Distillation is then discontinued.

After cooling, the flask is rotated to distribute the condensed fluxing agent homogeneously in the residue. When this is done the binder is ready for further examination.

Using a flask without a capillary and following this procedure, with domestic RC and MC cutbacks and SC road oils, the authors have obtained the results shown in Table VI. Figures are also given in Table VI for the separation of cutbacks into asphalts and fluxing agents using steam-distillation as described by Siegmann (*8*):

The distillation flask containing 600 grams of cutback is heated until the required temperature (Siegmann specifies 150° to 200° C. for gasoline; 200° to 220° C. for kerosene; 240° C. for gas oil) has been reached; then superheated steam of the same temperature is conducted through the mass in the flask. The distillation is continued until 10 times the amount of steam calculated on solvent (evaporation test) has passed over. It is of importance to ascertain whether, and how much, distillate passes over towards the end of the distillation. The best procedure is to plot a curve of the quantity of distillate against the quantity of water.

Though it is desirable to have an intake of 600 grams in order to make a reliable analysis of the separated solvent, in some cases, especially when analyzing road samples, the available quantity will be much smaller. In these cases the intake may be reduced to 300 grams, a 1-liter distillation flask being used instead of a 2-liter flask. If the cutback contains only a small percentage of volatile products, the total quantity of steam (10 times the amount of distillate) would be somewhat on the low side. Therefore, the minimum quantity of steam should in any case be not less than 200 grams.

Acknowledgment

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Physicochemical Assay of Vitamin A

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THE vitamin A potencies of various materials are determined in many laboratories by one or both of two well-known physicochemical methods. The method which is often considered to be the more exact depends upon the measurement of the absorption of ultraviolet light at the maximum (328 mμ) of the vitamin A absorption band of an alcohol solution of the material or a purified extract (*1*, *2*, *4*, *8*). The vitamin A concentration is proportional to the value of $E^{1\%}_{1\text{ cm.}}$ (328 mμ).

$$E^{1\%}_{1\text{ cm.}} = \frac{1}{cd} \log \frac{I_0}{I}$$

where c = concentration in grams per 100 ml., d = depth of optical cell in centimeters, I_0 = intensity of the light incident on the solution, and I = intensity of the light transmitted by the solution.

The other method, which is almost as exact, depends upon the measurement of the absorption of light at the maximum (620 mμ) of the absorption band of the blue colored reaction product formed by mixing a chloroform solution of the material with ten times its volume of a saturated chloroform solution of antimony trichloride (*3*, *5*, *6*). The vitamin A concentration is proportional to the value of $E^{1\%}_{1\text{ cm.}}$ (620 mμ). If a spectrophotometer is not available, this color measurement may be made with a photoelectric colorimeter using a sharp-cut filter. In this latter case a calibration curve is made relating the vitamin A potency of the solution being tested to the depth of the blue color.

TABLE I. STABILITY OF SOLUTIONS OF VITAMIN A KEPT IN BROWN GLASS BOTTLES

[Halibut liver oil distillate diluted with cottonseed (Wesson) oil]

Solvent	Location of Maximum Absorption	Concentration	$E^{1\%}_{1\text{ cm.}}$ (Max.) 1 hour	24 hours	7 days	50 days
	mμ	%				
Ethyl alcohol	328	0.0822	15.1	15.1	15.1	13.1
Cyclohexane	327	0.0863	14.6	15.0	14.6	14.5
Hexane	328	0.0844	16.1	15.8	15.6	16.0
Ether	328	0.0848	16.6	16.3	15.8	11.9
Chloroform	333	0.0846	13.2	13.6	14.4	13.2
Benzene	333	0.0846	14.4	14.3	14.3	14.7

When most fish liver oils and concentrates are tested by either of these methods the assays are precise and reproducible, especially in commercial laboratories doing the work as a routine procedure. The vitamin A solutions are prepared and tested within a half hour or, at most, an hour. However, the assays of low-potency fish liver oils, food products, and certain pharmaceutical preparations are not so satisfactory. These assays often involve extractions, saponification, etc., and take from 2 to 6 hours to complete. The potencies determined by these assays are often slightly, and many times surprisingly, low. The poor results are attributed to the instability of vitamin A in dilute solution. The degeneration of the vitamin is often assumed to be due to oxidation, but handling the solutions under inert gas does not improve the results to any great extent.

It has been shown (*7*) that vitamin A is destroyed by ultraviolet light, but it is not generally realized that the annoying instability of dilute solutions of vitamin A is almost entirely due to this effect. In Table I are given data which show the constancy for several days of the ultraviolet absorption of solutions of vitamin A stored in 50-ml. brown-glass-stoppered bottles. The solvents are of the ordinary C. P. grade, and the source of the vitamin A is a halibut liver oil distillate which had been diluted with cottonseed (Wesson oil) oil to a potency of slightly over 30,000 U. S. P. units per gram.

Since solutions of vitamin A are so stable in the absence of ultraviolet light, it seems desirable that they be handled entirely in apparatus which will absorb these harmful radiations. The apparatus, in general, must be transparent, so that the operator may separate layers, look for emulsion formation, etc.

Test tubes made of an amber glass which seemed suitable for the manufacture of such apparatus were furnished to the author by a glass manufacturer. Some tests of the usefulness of this glass were made.

Several solutions of vitamin A which were being assayed in the regular work were split up into two parts. One part was put into the amber test tube, and the other part into a clear test tube of the same dimensions. The tightly stoppered test tubes stood in a glass beaker on the laboratory bench 12 feet from the window of the room. After a few hours of this exposure to daylight, the solutions were again tested and the potencies of the vitamin A concentrates calculated. Cloudy days during which no sun shone were chosen for these tests, in order to help compensate for the higher actinic power of summer daylight. The solutions contained 20 to 25 units of vitamin A per ml.

Table II gives the results of these tests, which show that serious losses of vitamin A potency result from exposure of solutions of vitamin A in clear glass test tubes, while only small losses are found if amber test tubes are used. Contrary to prevalent opinions, solutions in chloroform are about as stable as solutions in alcohol, and solutions of vitamin A in the alcohol form are about as stable towards light as those in the ester form. This last fact should be borne in mind because, owing to the greater resistance of vitamin A esters to autoxidation, solutions of vitamin A esters are sometimes not handled so carefully as those of vitamin A alcohol.

TABLE II. EFFECT OF EXPOSURE TO LIGHT ON SOLUTIONS OF VITAMIN A

Material	Exposure to Daylight	Original Potency[a]	Solvent	Final Potency % by ultraviolet[a]	Final Potency % by $SbCl_3$[b]	Color of Test Tube
	Hours					
Vitamin A alcohol concentrate A, 8-1-40	5.5	3,260,000	EtOH	72	..	Clear
	Cloudy day			93	..	Amber
Vitamin A alcohol concentrate B, 9-4-40	5.0	2,989,000	EtOH	92	..	Clear
	Cloudy day			100	..	Amber
Vitamin A alcohol concentrate B, 8-28-40	4.0	2,980,000	$CHCl_3$	91	93	Clear
	Cloudy and rain			104	105	Amber
Fish liver oil C, 8-23-40	5.0	30,000	EtOH	45	..	Clear
	Cloudy day			96	..	Amber
Fish liver oil C, 8-23-40	4.0	30,000	$CHCl_3$	59	63	Clear
	Cloudy day			101	101	Amber
Fish liver oil distillate D, 8-27-40	5.0	216,000	EtOH	73	..	Clear
	Cloudy day			98	..	Amber
Fish liver oil distillate D, 8-27-40	4.0	216,000	$CHCl_3$	80	85	Clear
	Cloudy day			101	101	Amber

[a] Taken to be 2000 times $E^{1\%}_{1\text{ cm.}}$ (328 mμ) in alcohol.

[b] Measured with Evelyn photoelectric colorimeter using Rubicon 620 filter.

Unfortunately, no amber laboratory glassware is regularly made by the apparatus manufacturers. The author believes that every laboratory performing assays for vitamin A on food, medicinal, and physiological preparations needs at least the following types of amber glassware: separatory funnels, conical flasks, volumetric flasks, and boiling flasks for Soxhlet extractors.

There are undoubtedly other substances which could well be handled in amber glassware. The Kimble Glass Company, Vineland, N. J., has available the glass used in the work reported here, and is compiling a list of items which will be used in sufficient quantity to permit stocking. The coefficient of expansion is 64×10^{-7} per ° C., and apparatus made of it can be heated with reasonable care. The company will welcome suggestions concerning possible listings.

Acknowledgment

The author thanks George H. Wait for the experimental data given in Table II and the Eastman Kodak Research Laboratories for the optical measurements made for Table I.

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COMMUNICATION 20 from the laboratories of Distillation Products, Inc.

Determination of Small Amounts of Zinc in Plant Materials

A Photometric Dithizone Method

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DIPHENYLTHIOCARBAZONE, commonly referred to as "dithizone", has been used by several investigators (*2–4, 8*) as a colorimetric reagent for the determination of small amounts of zinc. The reaction between dithizone and zinc in slightly alkaline solution results in the formation of a red complex which is quantitatively extractable with chloroform or carbon tetrachloride. The reaction is extremely sensitive and the color sufficiently stable to form the basis of an excellent colorimetric method. However, more than a dozen other metals also react with dithizone to form extractable colored complexes. This lack of specificity has been the chief difficulty in the use of the reagent. It is possible to eliminate interferences to some extent by careful regulation of the pH at which the extractions are carried out and by the use of the selective complexing action of certain inorganic anions, but neither of these methods is entirely satisfactory.

Holland and Ritchie (*5*) reported the important observation, for which they give credit to R. H. Caughey, that in 0.02 *N* ammonium hydroxide solution sodium diethyldithiocarbamate, the copper reagent usually referred to as "carbamate", inhibits the reaction of all metals with dithizone except zinc. These workers proposed a colorimetric method for the determination of zinc in foods in which carbamate is used to eliminate interferences by other metals which form dithizone complexes. This method, although it represents an important step toward the solution of the problem, did not give highly reproducible results in the authors' hands when color intensities were measured with a photoelectric colorimeter. It was hoped by an investigation of the action of carbamate in the determination of zinc to develop a method free of interferences and capable of an accuracy comparable with that obtainable in measuring color intensities with modern photoelectric colorimeters.

Effect of Carbamate on Extraction of Zinc as Dithizonate

Zinc can be quantitatively extracted as dithizonate from aqueous solution at a pH between 8 and 9 with carbon tetrachloride containing excess dithizone. In the presence of carbamate, however, complete extraction of the zinc as dithizonate does not occur. The zinc is distributed between the red dithizone complex and the colorless carbamate complex. The result of this effect is a reduction in the color intensity of the dithizone extract, as is shown by a comparison of the curves on Figure 1.

Curve 1 represents the relationship between the amount of zinc present and the per cent light transmission of the carbon tetrachloride extract obtained when no carbamate is present, and curve 2 represents the same relationship when 12.5 mg. of carbamate are present. A comparison of the two curves shows that the presence of the carbamate appreciably reduces the color intensity of the carbon tetrachloride extract obtained for all amounts of zinc.

The effect of varying the amount of carbamate present

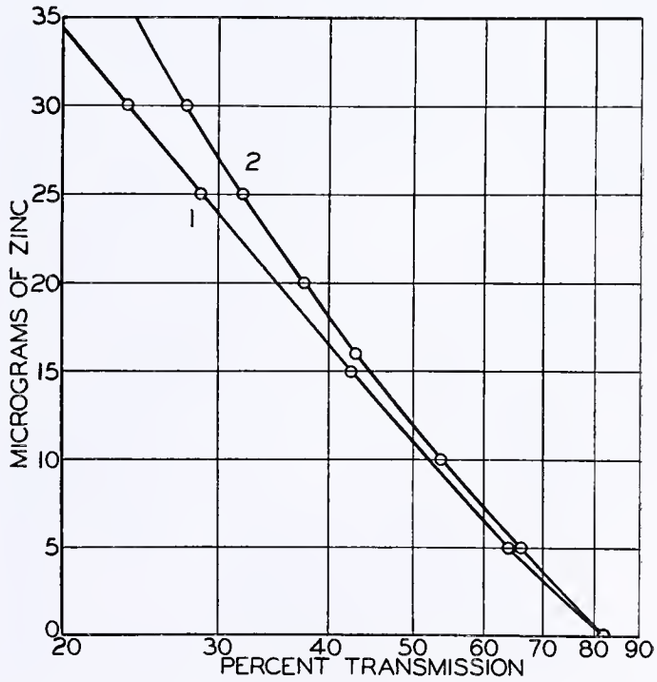

FIGURE 1. EFFECT OF CARBAMATE ON RELATION BETWEEN COLOR INTENSITY OF DITHIZONE EXTRACT AND AMOUNT OF ZINC PRESENT

1. No carbamate present
2. 12.5 mg. of carbamate present

Filter. Sextant green (3.52 mm.)
Cell thickness. 1 cm.

[1] Present address, American Viscose Company, Marcus Hook, Penna.

Table I. Effect of Amount of Carbamate Present on Distribution of Zinc between Dithizone and Carbamate Complexes

(30 micrograms of zinc present in each case)

Carbamate Present	Distribution: Dithizone		Distribution: Carbamate	
	γ	%	γ	%
Mg.				
0.00	30.0	100	0.0	0
0.63	28.9	96	1.1	4
1.25	29.0	97	1.0	3
2.5	28.1	94	1.9	6
5.0	27.3	91	2.7	9
12.5	25.9	86	4.1	14
25.0	21.9	73	8.1	27

Table II. Effect of Dithizone Complex-Forming Metals on Determination of Zinc

(10 micrograms of zinc present in each case)

Metal		Zinc Found	Error	
	γ	γ	γ	%
Cu^{++}	10	10.0	0.0	0
	20	10.1	0.1	1
	40	10.2	0.2	2
	60	10.3	0.3	3
	80	10.1	0.1	1
	100	10.2	0.2	2
Pb^{++}	20	10.3	0.3	3
	40	10.0	0.0	0
	60	10.2	0.2	2
	80	10.0	0.0	0
	100	10.4	0.4	4
Hg^{++}	100	10.1	0.1	1
Bi^{+++}	100	10.0	0.0	0
Co^{++}	100	10.2	0.2	2
Sn^{++}	100	9.9	−0.1	−1
Ni^{++}	100	10.6	0.6	6
Cd^{++}	5	10.4	0.4	4
	10	11.2	1.2	12
	20	12.2	2.2	22
	50	13.0	3.0	30
	100	16.7	6.7	67

upon the distribution of zinc between the carbamate and dithizone complexes is demonstrated by Table I. As the amount of carbamate is increased from 0 to 25 mg., the percentage of zinc extracted as dithizonate is decreased from 100 to 73 per cent.

In each of the extractions which were carried out in order to obtain the data for Figure 1 and Table I a fixed set of conditions was employed. The volume of the aqueous phase was 100 ml. and its pH 8.8. The aqueous phase, which contained the known amount of zinc, was then extracted with 10 ml. of dithizone reagent (about 0.10 gram per liter in carbon tetrachloride). The per cent transmission values were determined on 1 to 5 dilutions of the carbon tetrachloride extract with a Cenco-Sanford-Sheard photelometer using 1-cm. cells and a Sextant green (Corning No. 401) filter of a thickness of 3.52 mm. The excess dithizone was not removed from the extracts. Its green color was allowed to modify the color of the red zinc dithizonate. This so-called "mixed-color" method of color comparison has been used extensively in the determination of lead by dithizone (*1*, *6*).

As shown by Figure 1 and Table I, the extraction of zinc as dithizonate is appreciably affected by the presence of carbamate. However, it was found by repeated redeterminations of curve 2 that the relationship between the per cent light transmission and the amount of zinc present may be readily reproduced if all factors which affect the distribution of the zinc between the carbamate and dithizone complexes and the distribution of the excess dithizone between the aqueous and carbon tetrachloride phases are kept constant. These factors are the pH and volume of the aqueous phase, the amount of carbamate present, and the volume and concentration of the dithizone in carbon tetrachloride used. On the basis of this relationship a method was devised for the determination of traces of zinc in plant materials in which carbamate is used to eliminate the interference of other metals which also form dithizone complexes.

Outline of Method

1. Ashing of the sample and extraction of the zinc from the ash with hydrochloric acid.
2. Separation of the zinc and other metals which form dithizone complexes from the other constituents of the plant ash solution by repeated extraction with dithizone in carbon tetrachloride at a pH between 8.5 and 9 and in the presence of ammonium citrate to prevent the precipitation of iron and aluminum.
3. Separation of the zinc from copper and excess dithizone and solution of the zinc in a definite volume of standard acid by extraction of the dithizone extracts obtained in step 2 with 50 ml. of 0.02 *N* hydrochloric acid.
4. Extraction of the aqueous phase from step 3 with dithizone in carbon tetrachloride after the adjustment of the pH to a value between 8.5 and 9 with ammonia–ammonium citrate buffer and the addition of sodium diethyldithiocarbamate, employing in this extraction the same pH, volumes of phases, and amounts of carbamate and dithizone as were used in the determination of the standard curve. The amount of zinc present in the plant material is then obtained by comparison of the instrument reading of this extract with the standard curve.

Table III. Recovery of Added Zinc from Plant Materials

Material	Zinc Added	Zinc Present	Zinc Found	Error	
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	%
Dried grapes	0.0	11.4[a]	11.0	−0.4	−4
	0.0	11.4	11.4	0.0	0
	0.0	11.4	12.0	0.6	6
	0.0	11.4	11.4	0.0	0
	0.0	11.4	11.2	−0.2	−2
	10.0	21.4	22.0	0.6	3
	10.0	21.4	21.8	0.4	2
	20.0	31.4	31.4	0.0	0
	20.0	31.4	31.6	0.2	1
	20.0	31.4	30.8	−0.6	−2
	40.0	51.4	50.4	−1.0	−2
	40.0	51.4	51.8	0.4	1
	40.0	51.4	51.4	0.0	0
Dried asparagus	0.0	13.6[a]	13.6	0.0	0
	0.0	13.6	12.6	−1.0	−7
	0.0	13.6	13.8	0.2	2
	0.0	13.6	14.0	0.4	3
	0.0	13.6	14.0	0.4	3
	10.0	23.6	23.2	−0.4	2
	10.0	23.6	22.0	−1.6	−7
	10.0	23.6	23.6	0.0	0
	10.0	23.6	24.6	1.0	4
	20.0	33.6	32.6	−1.0	−3
	20.0	33.6	32.6	−1.0	−3
	20.0	33.6	34.2	0.6	2
	20.0	33.6	34.6	1.0	3
	40.0	53.6	53.6	0.0	0
	40.0	53.6	53.6	0.0	0
	40.0	53.6	53.4	−0.2	0
	40.0	53.6	53.6	0.0	0
Corn mea	0.0	9.3[a]	9.0	−0.3	−3
	0.0	9.3	9.4	0.1	1
	0.0	9.3	9.4	0.1	1
	0.0	9.3	9.4	0.1	1
	10.0	19.3	19.4	0.1	1
	10.0	19.3	19.4	0.1	1
	20.0	29.3	29.8	0.5	2
	20.0	29.3	29.8	0.5	2
	40.0	49.3	50.4	1.1	2
	40.0	49.3	49.0	−0.3	−1

[a] Zinc naturally present in material is assumed to be equal to average amount of zinc found by analysis by this method.

Reliability of Method

The reliability of this method was tested by studies involving (1) the ability to determine zinc accurately in the presence of other metals which form dithizone complexes, (2) the accuracy with which added zinc could be recovered from plant materials, and (3) the ability of the method to give good agreement between duplicate determinations on the same plant material. The results obtained in these three tests are given in Tables II, III, and IV, respectively.

Table II shows that the method is remarkably free of interferences by other metals which form dithizone complexes. It was found that zinc could be determined without serious interference in the presence of ten times as much copper, lead, mercury, bismuth, cobalt, tin, or nickel. The interference of cadmium was greater than any of the other metals studied; however, about half as much of this metal as zinc may be present without causing an appreciable error. Since this

Table IV. Zinc in Various Plant Materials

Material	Zinc Found: Sample A	Sample B	Av.	Deviation
	P. p. m.	*P. p. m.*	*P. p. m.*	%
Sugar-beet pulp	15.0	14.2	14.6	3
Oats	29.6	30.2	29.9	1
Wheat	29.4	31.0	30.2	3
Brome grass hay	20.6	20.4	20.5	0.5
Fresh alfalfa	32.4	31.8	32.1	1
Spinach[a]	92.0	92.0	92.0	0
Table beets	45.8	44.2	45.0	2
Onions	49.6	49.8	49.7	0
Parsnips	15.8	16.6	16.2	3
Celery	30.0	29.2	29.6	1
Carrots	23.0	24.2	23.6	2
Turnips[a]	84.4	85.0	84.7	0.5

[a] Zinc determined using 5-ml. aliquot of ash solution. A 10-ml. aliquot was used in all other cases. In every case a 5-gram sample of ground, air-dry material was ashed.

amount of cadmium is seldom encountered in the analysis of plant materials, its interference would rarely be significant.

Table III shows satisfactory recoveries of zinc added before ashing to dried grapes, dried asparagus, and corn meal.

Table IV shows that the method gives excellent agreement among duplicate determinations on the same material for a wide variety of plants. In this study two samples of each plant material were ashed, and the zinc was determined on one aliquot from each ash solution.

These three tests show that by the use of carbamate the interference of other metals in the determination of zinc is practically eliminated, and that the accuracy obtainable is consistent with the best photometric method for trace amounts.

The plant materials examined were dry-ashed and the zinc was extracted from the ash by means of hydrochloric acid. By means of the spectrograph Rogers and Gall (*7*) found that extraction of the ash of some plants with hydrochloric acid did not remove all the zinc. It is possible that this hydrochloric acid–insoluble zinc is from soil which contaminated the samples of plant material, or is zinc assimilated by the plant and either rendered insoluble by some reaction during ashing or occluded by hydrochloric acid–insoluble constituents of the ash. This problem is sufficiently important, particularly where plant materials containing large amounts of insoluble matter are analyzed, to warrant future attention.

Apparatus

All glass apparatus used in the application of this method must be carefully cleaned. All apparatus should be rinsed with strong acid, followed by four or five rinses with distilled water, and finally be given a rinse with zinc-free water (redistilled from Pyrex). Separatory funnels may be conveniently cleaned by first dipping them in warm chromic acid cleaning solution, which is removed by three thorough rinsings with tap water. The funnels are then rinsed with distilled water four times, and given a final rinse with zinc-free water. Care must be taken that no chromic acid remains on the funnels, for it will cause trouble by oxidizing dithizone.

All vessels in which aqueous solutions are stored for more than a few hours should be of Pyrex glass. Soda-lime separatory funnels, volumetric flasks, etc., in which solutions are kept for only a short time, were used and found satisfactory. White petroleum jelly was found to be a satisfactory lubricant for the stopcocks of the separatory funnels. A separatory funnel rack is necessary. Convenient racks have been described by Winter *et al.* (*9*) and by Bambach (*1*).

Although a Cenco-Sanford-Sheard photoelectric colorimeter was used exclusively in this investigation, the method is adaptable to any good photoelectric colorimeter.

Reagents

All water used in the preparation of the ash solutions and of reagents should be redistilled from an all-Pyrex glass still, unless it is definitely known that the distilled water available is free of zinc and other dithizone complex-forming metals. The distilled water available in this laboratory did not require redistillation. C. P. carbon tetrachloride may be used without purification. Technical carbon tetrachloride, however, should be dried with anhydrous calcium chloride and redistilled in the presence of a small amount of calcium oxide before use. Used carbon tetrachloride may be reclaimed by distillation in the presence of dilute sodium hydroxide solution containing a small amount of sodium thiosulfate, followed by drying with anhydrous calcium chloride and fractional distillation in the presence of a small amount of calcium oxide.

Most of the necessary reagents contain appreciable amounts of zinc and other metals that form dithizone complexes, which must be removed if good accuracy is expected by use of this method. Chemicals of the highest grade should be used and purified as follows:

Standard Zinc Solutions. Stock solution (1000 micrograms of zinc per ml.). Place 0.25 gram of pure zinc in a 250-ml. volumetric flask. Add about 50 ml. of water and 1 ml. of concentrated sulfuric acid, then heat on the steam bath until all zinc is dissolved. Dilute to 250 ml. and store in a Pyrex vessel.

Standard solution (10 micrograms of zinc per ml.). Dilute 10 ml. of the above stock solution to 1 liter. Store in a Pyrex vessel.

N Ammonium Hydroxide Solution. Distill into water one-half volume of concentrated ammonium hydroxide by use of an all-Pyrex glass distillation apparatus, then dilute to the proper concentration. Store in a glass-stoppered Pyrex vessel.

N Hydrochloric Acid. Displace hydrogen chloride gas from a volume of concentrated hydrochloric acid in a Pyrex flask by the slow addition of an equal volume of concentrated sulfuric acid by means of a dropping funnel which extends below the surface of the concentrated hydrochloric acid. Absorb the displaced hydrogen chloride gas by conducting it by means of a delivery tube to the surface of a volume of water. No heat is necessary. Dilute to the proper concentration. One hundred and fifty milliliters of each of the acids will yield 1 liter of purified hydrochloric acid solution of a concentration greater than normal.

Dithizone Reagent. Dissolve 0.20 gram of diphenylthiocarbazone in 500 ml. of carbon tetrachloride and filter the solution to remove insoluble matter. Place the solution in a glass-stoppered bottle or large separatory funnel, add 2 liters of 0.02 *N* ammonia (40 ml. of *N* ammonia diluted to 2 liters), then shake to extract the dithizone into the aqueous phase. Separate the phases, discard the carbon tetrachloride phase, and extract the ammoniacal solution of dithizone with 100-ml. portions of carbon tetrachloride until the carbon tetrachloride extract is a pure green color. Discard the carbon tetrachloride phase after each extraction. Add 500 ml. of carbon tetrachloride and 45 ml. of *N* hydrochloric acid and shake to extract the dithizone into the carbon tetrachloride. Separate the phases and discard the aqueous phase. Dilute the carbon tetrachloride solution of dithizone to 2 liters with carbon tetrachloride. Store the dithizone reagent in a brown bottle in a dark cool place.

Ammonium Citrate Solution (0.5 molar). Dissolve 226 grams of ammonium citrate, $(NH_4)_2HC_6H_5O_7$, in 2 liters of water. Add concentrated ammonia (80 to 85 ml.) until the solution has a pH of 8.5 to 8.7. Add an excess of dithizone reagent (orange-yellow coloration in aqueous phase after shaking and separation of phases), and extract with 100-ml. portions of carbon tetrachloride until the extract is a full green color. Add more dithizone if necessary. Separate the aqueous phase from the carbon tetrachloride, and store in a Pyrex vessel.

Carbamate Reagent. Dissolve 0.25 gram of sodium diethyldithiocarbamate and dilute to 100 ml. with water. Prepare a fresh solution just before use.

Three solutions are prepared in large quantities from the above reagents in order to reduce the measuring out of reagents and to minimize errors due to variations in their composition. These solutions should be stored in Pyrex vessels, and care must be taken to avoid loss of ammonia from Solutions A and B. The amount of zinc in these reagents increases slowly with time of storage, and they should be discarded after standing 6 to 8 weeks. A standard curve must be determined for each new set of reagents. The amounts of reagents designated below and 2 liters of dithizone reagent are sufficient for 100 zinc determinations.

Solution A. Dilute 1 liter of 0.5 *M* ammonium citrate and 140 ml. of *N* ammonium hydroxide to 4 liters.

Solution B. Dilute 1 liter of 0.5 *M* ammonium citrate and 300 ml. of *N* ammonium hydroxide to 4.5 liters. Just before use add 1 volume of freshly prepared carbamate reagent to 9 volumes of the ammonia–ammonium citrate solution to obtain the volume of Solution B immediately required.

0.02 *N* Hydrochloric Acid. Dilute 100 ml. of *N* hydrochloric acid to 5 liters.

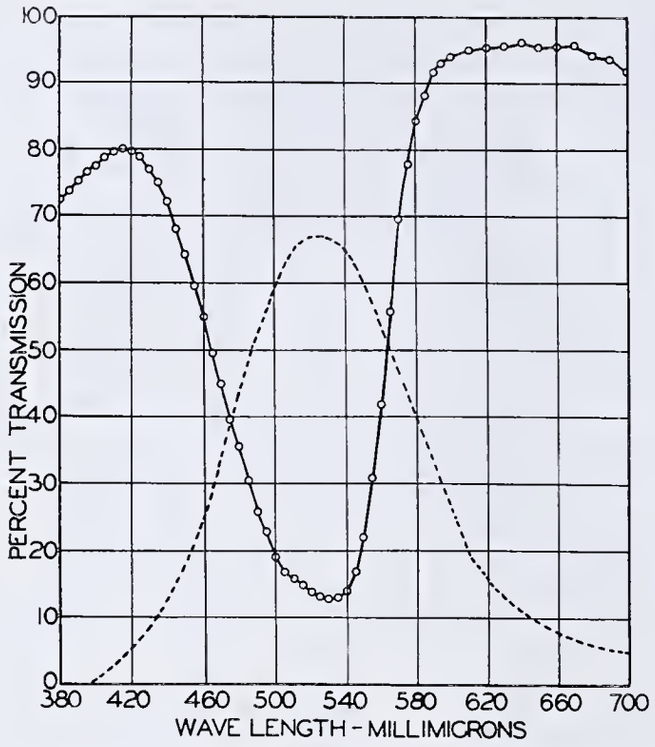

FIGURE 2. LIGHT TRANSMISSION OF SEXTANT GREEN FILTER AND ZINC DITHIZONE COMPLEX

Broken line. Sextant green filter
Solid line. Zinc dithizonate in carbon tetrachloride

Once a set of reagents purified from zinc has been prepared, these reagents can be used to test chemicals for zinc. Certain lots of ammonium hydroxide and hydrochloric acid are sufficiently free of zinc to be used in this method without purification.

Procedure for Zinc in Plant Materials

ASHING. Ash a 5-gram sample of the finely ground, air-dry plant material in a platinum dish in an electric muffle at 500° to 550° C. Wet the ash with a little distilled water, then add 10 ml. of *N* hydrochloric acid (more if necessary) and heat on a steam bath until all substances soluble in hydrochloric acid are brought into solution. Add 5 or 10 ml. of hot water. Filter off the insoluble matter on a 7-cm. filter paper (Whatman No. 42 or equivalent) which has been previously washed with two 5-ml. portions of hot *N* hydrochloric acid, then washed with hot water until free of hydrochloric acid, and collect the filtrate in a 100-ml. volumetric flask. Wash the filter with hot water until washings are no longer acid to methyl red. Add a drop of methyl red indicator to the filtrate in the 100-ml. flask, add *N* ammonium hydroxide until neutral to methyl red, then add 4 ml. of *N* hydrochloric acid. Allow the contents of the flask to cool, then make to volume with water.

FIRST EXTRACTION (separation of dithizone complex-forming metals from the ash solution). Pipet an aliquot of the ash solution containing not more than 30 micrograms of zinc into a 125-cc. Squibb separatory funnel. Add 1 ml. of 0.2 *N* hydrochloric acid for each 5 ml. of ash solution less than 10 ml. taken, or 1 ml. of 0.2 *N* ammonium hydroxide for each 5 ml. over 10 ml. taken. A 10-ml. aliquot has usually been found satisfactory in the analysis of plant materials. Add 40 ml. of Solution A and 10 ml. of dithizone reagent. Shake vigorously for 0.5 minute to extract from the aqueous phase the zinc and other dithizone complex-forming metals which may be present, then allow the layers to separate. At this point an excess of dithizone, which is indicated by an orange or yellow-orange coloration of the aqueous phase, must be present. If excess dithizone is not present, add more of the reagent until after shaking an excess is indicated.

Shake down the drop of carbon tetrachloride extract from the surface, and draw off the carbon tetrachloride extract into a second separatory funnel as completely as possible without allowing any of the aqueous layer to enter the stopcock bore. Rinse down the carbon tetrachloride extract from the surface of the aqueous layer with a 1- to 2-ml. portion of clear carbon tetrachloride, then run off this carbon tetrachloride into the second funnel without permitting the aqueous phase to enter the stopcock bore. Repeat this rinsing process as many times as is necessary to flush the extract completely into the second funnel. Add 5 ml. of clear carbon tetrachloride to the first funnel, shake 0.5 minute, and allow the layers to separate. The carbon tetrachloride layer at this point will have a clear green color if metals which form dithizone complexes have been completely extracted from the aqueous phase by the previous extraction. Run off the carbon tetrachloride layer into the second funnel, then flush down the extract from the surface and out of the funnel as was done before. If the last extract does not possess a distinct clear green color, repeat the extraction with a 5-ml. portion of clear carbon tetrachloride and the flushing out process until complete extraction of the dithizone complex-forming metals is assured, then discard the aqueous phase.

SECOND EXTRACTION (separation of copper by extraction of zinc into 0.02 *N* hydrochloric acid). Pipet 50 ml. of 0.02 *N* hydrochloric acid into the separatory funnel containing the carbon tetrachloride solution of metal dithizonates. Shake vigorously for 1.5 minutes, then allow the layers to separate. Shake down the drop from the surface of the aqueous phase, and run off as completely as possible the carbon tetrachloride phase, which contains all the copper as dithizonate, without allowing any of the aqueous phase, which contains all the zinc, to enter the stopcock bore. Rinse down the carbon tetrachloride extract from the surface of the aqueous phase and rinse out the stopcock bore with 1- to 2-ml. portions of clear carbon tetrachloride (in the same manner as was done in the first extraction) until all traces of green dithizone have been washed out of the funnel. Shake down the drop of carbon tetrachloride from the surface of the aqueous phase, and run off the carbon tetrachloride as completely as possible without allowing any aqueous phase to enter the stopcock bore. Remove the stopper from the funnel and lay it across the neck of the funnel until the small amount of carbon tetrachloride on the surface of the aqueous phase has evaporated.

FINAL EXTRACTION (extraction of zinc in the presence of carbamate reagent). Pipet 50 ml. of Solution B and 10 ml. of dithizone reagent into the 50 ml. of 0.02 *N* hydrochloric acid solution. Shake for one minute, then allow the phases to separate. Flush out the stopcock and stem of the funnel with 1 ml. or so of the carbon tetrachloride extract, then collect the remainder in a test tube. Pipet 5 ml. of the extract into a 25-ml. volumetric flask and dilute to the mark with clear carbon tetrachloride, and then determine the per cent light transmission of the diluted solution with a photoelectric colorimeter, equipped with a Sextant green (Corning No. 401) filter, or equivalent. Readings should be taken not later than 2 hours after the final extraction. Protect extracts from light as much as possible.

A blank determination must be run with each series of zinc determinations in order to determine the correction to be applied for the zinc present in the hydrochloric acid and ammonium hydroxide in the ash solution and the zinc picked up by the other reagents from their containers since the determination of the standard curve. The blank determination is carried out exactly as is done in the case of the plant material, using as a starting point an empty platinum dish that is placed in the muffle along with the samples of plant material.

The amount of zinc on the standard curve corresponding to the per cent light transmission obtained for the unknown less the amount of zinc corresponding to the per cent transmission obtained for the reagent blank gives the amount of zinc present in the aliquot. From this the amount of zinc in the sample is easily calculated. As many as 12 determinations may conveniently be carried simultaneously through this procedure.

Standard Curve

The data for the standard curve are obtained by determining the per cent transmission values for each of a series of solutions containing known amounts of zinc. These zinc solutions are prepared as follows:

Place 0, 5, 10, 15, 20, 25, 30, and 35 ml. of the standard zinc solution containing 10 micrograms of zinc per ml. in 100-ml. volumetric flasks. To each add one drop of methyl red indicator, *N* ammonium hydroxide until neutral, and 4 ml. of *N* hydrochloric acid, and make to volume. Proceed from this point in exactly the same manner as in the case of ash solutions, beginning with the first extraction and using 10-ml. aliquots of each of the zinc solutions. The 10-ml. aliquots contain 0, 5, 10, 15, 20, 25, 30, and 35 micrograms of zinc, respectively.

The standard curve is constructed by plotting micrograms of zinc against per cent light transmission.

A One-Color Method

An investigation was carried out to determine whether the method was reliable if the excess dithizone was removed from the final carbon tetrachloride extract, and the color comparisons were made on the red zinc dithizonate solutions obtained. The color comparisons, if the method proved satisfactory, could then be made conveniently by use of either an ordinary visual colorimeter or a photoelectric colorimeter. It was found that the excess dithizone could be readily removed from the final dithizone extract without appreciable loss of zinc by a wash with 50 ml. of 0.01 *N* ammonia, and that the per cent transmission of the solutions obtained was a reproducible function of the amount of zinc present. Copper was not found to interfere when present in amounts up to 100 micrograms, but metals which are extracted along with the zinc into the 0.02 *N* hydrochloric acid solution, such as lead, were found to interfere. It seems that during the removal of the excess dithizone the colorless lead carbamate complex present in the carbon tetrachloride extract is decomposed.

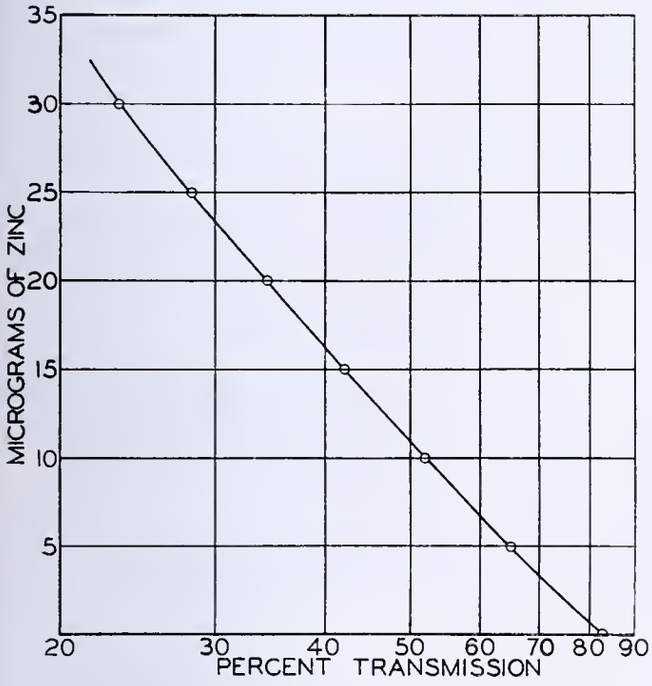

FIGURE 3. STANDARD CURVE OF RELATIONSHIP BETWEEN ZINC PRESENT AND PER CENT TRANSMISSION OF DITHIZONE EXTRACT

Filter. Sextant green (6.00 mm.)
Cell thickness. 1 cm.

The lead then combines with dithizone and forms its red dithizone complex, which causes high results. The one-color method was therefore considered inferior to the mixed color method because of these interferences.

Choice of Color Filter

The light transmission of zinc dithizonate (0.6 mg. of zinc per liter of carbon tetrachloride) for different wave lengths of light was determined by use of a Cenco spectrophotelometer, and is represented by the solid curve on Figure 2. This curve shows that zinc dithizonate has a narrow absorption band between 450 and 575 microns with a maximum absorption at about 535 microns. It is therefore necessary to use in the photoelectric colorimeter a filter that transmits a narrow band of light in this same region of the spectrum, in order to obtain a maximum sensitivity of the instrument to differences in concentration of zinc dithizonate in the solutions compared. These requirements seem to be met best by the Sextant green (Corning No. 401) filter. The light transmission characteristics of this filter (1-mm. thickness) are represented by the dotted curve on Figure 2. A comparison of the two curves indicates the close coincidence of the region of light transmission of this filter with the region of light absorption by the zinc dithizonate solution.

In the preliminary investigation a filter of 3.52-mm. thickness was used. It was found that even greater sensitivity could be obtained by using both the 3.52-mm. and a 2.48-mm. filter, which gave a total filter thickness of 6.00 mm. The standard curve obtained using both Sextant green filters of a total thickness of 6.00 mm. is shown on Figure 3, where per cent transmission is plotted as a function of the amount of zinc present for 1-cm. cells by the mixed-color method. This curve and filter were used in obtaining the data given in Tables II, III, and IV.

Other Applications of Method

Although this method for zinc has been applied here only to the analysis of plant materials, there is no apparent reason why it should not serve equally well for the determination of small amounts of zinc in many other types of materials from which the zinc present may be obtained in solution. If less than one half as much cadmium or less than ten times as much of other dithizone complex-forming metals as zinc are present and substances are not present which are not kept in solution by citrate during the first extraction, one should be able to apply the method directly. Excessive amounts of other dithizone complex-forming metals would be troublesome because of the large volume of dithizone extract that would be obtained in the first extraction. Should trouble be encountered for any of these reasons, a preliminary separation of the substances causing the difficulty would be necessary.

Summary

A photometric, "mixed-color" dithizone method has been developed for the determination of zinc in plant materials in which sodium diethyldithiocarbamate is used to eliminate the interference of other metals which form colored complexes with dithizone. It was found that "carbamate" causes an appreciable reduction in the color intensity of the dithizone extract, but, by keeping conditions constant in all extractions, a reproducible relationship is obtainable between the color intensity of the dithizone extract as measured with a photometric colorimeter and the amount of zinc present.

Tests involving the determination of zinc in the presence of other metals which form dithizone complexes, the recovery of added zinc from various plant materials, and the agreement between duplicate determinations proved the method to be accurate and remarkably free of interferences.

A one-color method for the determination of zinc was tested and found to be inferior to the mixed-color method.

The light transmission curve of zinc dithizonate in carbon tetrachloride was determined with a spectrophotometer. This curve is discussed in relation to filter selection for the photometric determination of zinc with dithizone.

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Determination of Total Vitamin A Content of Dairy Butters

Spectrophotometric Method

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AN ACCURATE and relatively rapid method for the determination of the total—(i. e., carotene plus vitamin A)—vitamin A potency of dairy butter has been desirable, in order to make a more thorough study of the range of vitamin A potency in this food product. It is generally recognized that the biological method is of only limited accuracy, with rather wide variations in results to be expected (*2*).

Literature Review

A review of the literature bearing on the subject reveals that many methods involving various techniques and instruments have been used by different investigators in an attempt to determine the vitamin A potency of dairy butter by physical methods.

Fraps and Kemmerer (*5*) have estimated the vitamin A in butter by determining the absorption at 328 millimicrons (corrected for carotene absorption) of a methanol solution of the unsaponifiable residue, after chilling out light-absorbing impurities, and estimating the carotene content by colorimetric comparison against potassium bichromate. The authors' experience with this method has led them to believe that it gives high results, probably because the chilling does not remove all the nonvitamin material which absorbs light at 328 millimicrons. And, of course, it would be advantageous to determine both vitamin A and carotene in one operation.

Gillam, Heilbron, Morton, Bishop, and Drummond (*7*) attacked the problem by separating the carotene and xanthophyll, and determining these substances separately by spectrographic means, then determining vitamin A by its absorption at 328 millimicrons in the presence of carotene in one fraction and of xanthophyll in the other fraction (correcting for carotenoid absorption). This method is time-consuming and does not take into consideration the natural unsaponifiable materials in oils and fats which absorb some light in the region of 328 millimicrons, but which possess no vitamin A activity. A short time later, Gillam (*6*) improved and shortened the method by showing that about 94 per cent of the light absorption at 455 to 460 millimicrons (in chloroform) is due to carotene, and that the absorption at 328 millimicrons, due to carotene plus xanthophyll, can be corrected for by a constant factor. However, there still remained the problem of accounting for other unsaponifiable materials of no vitamin A potency, which do have general absorption in the region of 328 millimicrons, and hence give high results.

Baumann and Steenbock (*3*) determined carotene and vitamin A separately. For carotene, the absorption values were measured at 460 and 485 millimicrons on melted whole butterfat, and for calculation they compared these values with the absorption of standard solutions of carotene in refined cottonseed oil. The authors' experience over a number of years has indicated that the glyceride composition, free fatty acid content, color components, and other factors such as unsaponifiable content both of refined cottonseed oil and of butterfat are very variable; hence, results which do not take these factors into account may be somewhat variable. Vitamin A was determined on the butterfat unsaponifiable, in methyl alcohol solution, by determining the absorption at 328 millimicrons after first purifying by chilling at −72° C. in a solid carbon dioxide–acetone mixture. These workers recognized that at times excess absorption was found at 328 millimicrons; and attempted to minimize errors that might otherwise be thus introduced by discarding all results in which they found that the absorption at 280 millimicrons exceeded that at 328 millimicrons. However, it is evident from a study of their work that an "actual" control for the spectrophotometric determination of carotene and vitamin A in dairy butter should be very helpful.

Experimental

Attempts were made to determine spectrophotometrically the vitamin A and carotene in whole butterfat, by first removing the vitamin materials by the use of adsorbents and using the devitaminized oil as a control for the untreated oil for direct spectral determination in a suitable solvent. Kraybill and Shrewsbury (*8*, *9*) have shown that charcoal and Lloyd's reagent remove practically all the vitamin A and carotene material in butterfat. However, the authors found that these adsorbents remove other nonvitamin constituents from the control oil sample; hence, the method gave high results and was discarded.

From all the above, it appeared that the most progress could be made by working on the unsaponifiable fraction of butterfat. The problem then resolved itself into three phases: (1) development of a satisfactory technique for the extraction of the unsaponifiable material containing all the vitamin A and carotene present in the original samples, (2) selection of a satisfactory solvent for the unsaponifiable fraction, and (3) obtaining an "actual" control, containing all of the constituents of the unsaponifiable material of the particular sample under test except vitamin A and carotene; and using this control for spectrophotometric determination of the total vitamin A (plus carotene) content of the sample.

In their work, the authors used cyclohexane specified as "purified for spectrophotometric use, free of extraneous ultraviolet absorption". This solvent has been shown by the above technique to be satisfactory in every instance except one. In this instance, the authors were able satisfactorily to purify a shipment of cyclohexane which showed extraneous ultraviolet absorption, by the following treatment, carried out in all-glass equipment: Let the cyclohexane stand, with intermittent shaking, in contact with 10 per cent by weight of concentrated sulfuric acid, for a period of one week, and at a temperature of about 21° to 27° C. (70° to 80° F.); decant the greater part of the solvent from the acid, and distill through a fractionation column.

The most difficult problem encountered in connection with the extraction of unsaponifiable material was that of obtaining solvents in sufficiently pure form. It has been the authors' experience that the ether and alcohol solvents used for this purpose must be carefully purified immediately before use, in order to avoid oxidation of the unsaponifiable material. Details of the purification processes used for these solvents are given under "Method".

Of the optically satisfactory solvents, cyclohexane was found to be the most satisfactory because it is a good solvent for fat and unsaponifiable matter under the conditions employed, it is satisfactory from the standpoint of volatility, and vitamin A and carotene have been demonstrated, in this laboratory, to be stable in a cyclohexane solution for several days, provided that the solutions are stored in the dark and that the cyclohexane is exceedingly pure. It has been the authors' practice to purchase cyclohexane which has been specially purified for spectrophotometric purposes; before using, they compare this solvent spectrophotometrically with a sample known to be satisfactory. Cyclohexane which shows no extraneous absorption in the region between

5000 and 2200 Å. is considered to be sufficiently pure for use; while any sample which shows extraneous absorption within this region is rejected as being impure.

It is well known that vitamin A and carotene are destroyed by ultraviolet light. Demarest (*4*) published the results of his studies on vitamin A determination by destructive irradiation while this present work was in progress.

Preliminary work showed that the vitamin A content of whole butterfat could not successfully be destroyed by ultraviolet light irradiation (from any source of ultraviolet at the authors' command), probably because of the protection afforded to the vitamin compounds by the glycerides of the whole oil. However, it was found that the vitamin A and carotene could be successfully destroyed by ultraviolet irradiation of a cyclohexane solution of the unsaponifiable extract from butterfat. This irradiated solution, containing all of the unsaponifiable fractions of the sample under test (except the vitamin A, carotene, and xanthophyll which have been destroyed by the irradiation), serves as a satisfactory control for the spectrophotometric measurements of the vitamin A and carotene in another portion of the same original cyclohexane solution of the unsaponifiable matter which has not been subjected to the action of ultraviolet light.

The following method, then, is based on the destruction of vitamin A and carotene in a portion of a cyclohexane solution of the unsaponifiable fraction of butterfat, and the use of this "devitaminized" solution as a control for the simultaneous spectrophotometric determination of both vitamin A and carotene in a second portion of the original unsaponifiable solution not exposed to the action of ultraviolet light.

Equipment Used

Spectrophotometer. Adam Hilger, Ltd., intermediate quartz spectrograph with Spekker photometer, equipped with tungsten steel electrodes as a source of light.

Quartz absorption cells, Hilger Type C, 1-cm. Quartz Kjeldahl-shaped flasks, 25-cc.

Ultraviolet lamp. Mercury lamp, rectifier-type quartz arc operated to be equivalent to a 250-watt direct current Uviarc: Uviarc poultry treater, Type RT, Spec. 100, Cooper Hewitt Electric Co., Hoboken, N. J. Operated through an alternating current auxiliary, Code No. 260, 7A4 × 4, 186 to 254 volts, 60 cycles, 2.4 amperes, 450 watts, made by Cooper Hewitt Electric Co., Hoboken, N. J. (The spectral radiation for this lamp can be obtained by taking three fourths of the values given in column 1—i. e., for the 120-volt direct current Uviarc—of data sheet S-201 compiled by the Commercial Engineering Department, General Electric Vapor Lamp Co., Hoboken, N. J.)

Method

Extraction of Unsaponifiable Material. Melt the butter in a water bath at about 60° C., and separate the fat by filtration through a Whatman No. 12 folded filter paper.

Saponify 25 grams of the fat with 30 cc. of a 20 per cent solution of potassium hydroxide in alcohol (specially denatured No. 30) by boiling, with a suitable reflux arrangement, for 5 minutes only.

Dilute the alcoholic solution with water to approximately four volumes and cool in an ice-water bath. Then extract the unsaponifiable matter with cold ethyl ether (usually four extractions with the following successive amounts of ether are sufficient: 180, 150, 100, 50 cc.). Vigorous shaking is required for complete extraction of the unsaponifiable material.

Composite the ether extracts and wash with 150-cc. portions of distilled water until substantially free of alkali, taking due care to avoid troublesome emulsions. Usually six washings are sufficient. The first two washings should be made by merely pouring the water through the ether without shaking.

Filter the ether solution through filter paper and concentrate to 25 to 50 cc. by distillation on a steam bath. Remove the remainder of the ether by evaporating, on a steam bath, under a stream of carbon dioxide to prevent oxidation. When substantially all the ether has been evaporated, cool immediately to about 21° C. (70° F.), and dissolve the unsaponifiable residue in cyclohexane. Make the cyclohexane solution up to 50 cc. (50 per cent solution, weight to volume, on original oil basis). Filter and store in the dark at about 4° to 10° C. (40° to 50° F.) until the sample is examined spectrophotometrically. (The sample should not be stored more than 2 days before its vitamin content is determined.)

Note. Both the ether and alcohol must be carefully purified immediately before using. This is satisfactorily accomplished in the case of ethyl ether (anhydrous c. p.) by letting the ether stand with intermittent shaking, for 3 hours, over a 5 per cent aqueous potassium hydroxide solution, then slowly distilling. The alcohol (specially denatured No. 30) is satisfactorily purified by treating with potassium hydroxide and aluminum grains followed by distillation (*1*).

Spectrophotometric Determination of Carotene and Vitamin A. Divide the cyclohexane solution of unsaponifiable material in two parts, and irradiate one portion, contained in a 25-cc. Kjeldahl-shape quartz flask, under the Uviarc as follows (allowing at least 10 minutes for the lamp to come to full operating temperature before starting irradiations): Stopper the flask with a cork wrapped in aluminum foil, and support the flask in such a position that the cork rests against the rim of the lamp and the bulb of the flask is held about 10 cm. (4 inches) away from the mercury tube of the lamp. Place a sheet of aluminum foil about 7.5 cm. (3 inches) below the flask being irradiated (to reflect the light back toward the sample). Agitate the sample every 15 minutes by gently tapping the flask (for example, with a pencil). Allow the sample to heat up as much as the lamp will heat it, provided that the temperature of the sample does not reach the boiling point of cyclohexane.

Irradiate until the vitamin A and carotene have been destroyed. Under the authors' conditions, 1 to 1.5 hours has proved sufficient time for irradiation. Destruction of carotene and vitamin A can be estimated by the disappearance of the carotene yellow color, and by the Carr-Price test.

After cooling to about 21° C. (70° F.), filter the irradiated solution, which must be clear and colorless, and determine carotene and vitamin A in the nonirradiated solution by means of the spectrophotometer (1-cm. cells) using as a control the ultraviolet irradiated solution. Expose the plates at density settings ranging from 0 to 1.50 in increments of 0.05, with the exposure time graduated up to about two seconds on Eastman 33 plates (D 72, diluted 1 to 2, is a satisfactory developer for these plates).

Read the carotene and vitamin A match points from the same plate. The vitamin A is read at 328 millimicrons (3280 Å.) and the carotene at 460 millimicrons (4600 A.). (These wave lengths as points of maximum absorption are in agreement with published works, *6*, and have been verified in this laboratory for solutions of vitamin A concentrates and carotene in cyclohexane.) Of course, the difference in absorption between the nonirradiated and the irradiated sample, at the wave lengths indicated above, is a measure of the vitamin A and carotene content of the sample.

Calculations. *I. Carotene in Terms of Vitamin A.*

Match point at 4600 A. × 0.94 = corrected match point at 4600 A.

$$\frac{\text{Corrected match point at 4600 Å.} \times 4.54 \times 1000}{0.50 \times 2.1 \times 0.6} = \text{U. S. P. XI vitamin A units per pound of butterfat, due to carotene}$$

Based on:

1. 94 per cent of the light absorption at 4600 Å. is due to carotene (*6*).
2. $E_1^{1\%}{}_{\text{cm.}}$ at 4600 Å. for carotene in cyclohexane = 2100. Established for the authors' instrument by the use of a sample of β-carotene containing about 10 per cent of α-carotene, obtained from the S. M. A. Corp., Cleveland, Ohio.
3. By definition, 0.6 microgram of β-carotene equals one International unit of vitamin A (*10*).

II. Vitamin A.

$$\text{Match point at 3280 Å.} - \frac{\text{match point at 4600 Å.}}{6.5} = \text{corrected match point at 3280 Å.}$$

$$\frac{\text{Corrected match point at 3280 Å.}}{0.50} \times (2140 \times 4.54) = \text{U. S. P. XI vitamin A units per pound of butterfat, due to vitamin A}$$

Based on:

1. The absorption at 328 millimicrons due to carotene plus xanthophyll is given by dividing the observed value of $E_1^{1\%}{}_{\text{cm.}}$ for these substances at 455 to 460 millimicrons (*6*) by the factor 6.5.

TABLE I. ESTABLISHMENT OF INSTRUMENT FACTOR

(Used at 2140)

Date of Determination	Per Cent Solution (Original Oil Basis)	Match Point at 3280 Å.	$E^{1\%}_{1\text{ cm.}}$ Value	Instrument Factor
2–20–39	0.7000	0.98	1.400	2143
2–24–39	0.7000	0.98	1.400	2143
2–27–39	0.7000	0.99	1.414	2122
3– 4–39	0.8000	1.12	1.400	2143
3– 6–39	0.9000	1.25	1.389	2159
3–13–39	0.7000	0.98	1.400	2143
			Av.	2142

2. 2140 as the instrument factor for vitamin A.

Total. I + II = total U. S. P. units of vitamin A per pound of butterfat due to both carotene and vitamin A.

Notes. The above correction factors have been accepted from the literature (*6*) without verification in the laboratory because they are considered as average figures, and any probable individual variation from these average figures would result in only minor differences in the final results.

As a check on the above irradiation procedure, the authors have adopted the following practice:

A second spectrophotometric determination is made on each (nonirradiated) unsaponifiable solution; however, pure cyclohexane is used instead of the above irradiated solution of the unsaponifiable as a control for this (second) determination. The match point at 3280 Å. is compared with that obtained in the original determination. Of course, the match point obtained by the use of the pure cyclohexane control is numerically greater than that obtained by the use of the irradiated unsaponifiable solution as a control. The results of approximately 100 determinations have indicated that this difference is in the range of 10 to 25 per cent. Any difference greater than 25 per cent between the match points (at 3280 Å.) obtained from the cyclohexane and from the irradiated control would indicate the possibility of incomplete destruction of vitamin materials in the irradiated sample, oxidation, or evaporation of solvent, and would necessitate repetition of the determination.

DERIVATION OF INSTRUMENT FACTOR OF 2140. The instrument factor for converting from the $E^{1\%}_{1\text{ cm.}}$ value for vitamin A, read at 3280 Å. units, to U. S. P. units of vitamin A per gram, was determined for the authors' instrument from the U. S. P. standard of reference cod liver oil.

Results of several instrument factor determinations are listed in Table I. In these determinations the authors used U. S. P. reference cod liver oil containing 3000 U. S. P. XI vitamin A units in 1 gram of the oil. Distribution date was 11–29–38, control 2-38-A, not to be used after 5–29–39.

Note. The method used for the determination of the instrument factor is as follows (the saponification and extraction procedure is a modification of the procedure published by Wilkie, *11*):

Saponify 0.7 to 1.0 gram of U. S. P. standard of reference cod liver oil in a 250-cc. Erlenmeyer flask with 3 cc. of a 50 per cent aqueous solution of potassium hydroxide, after the addition of 30 cc. of purified (see method above) specially denatured alcohol No. 30, by heating on a hot plate for exactly 3 minutes. Suitable reflux arrangements must be provided to prevent loss of solvent during saponification.

Add 20 cc. of distilled water and cool by placing the flask in ice water for 10 minutes.

Transfer the solution to a 250-cc. pear-shaped separatory funnel; extract the unsaponifiable material by shaking vigorously for 1 minute each time, with three 50-cc. portions of freshly purified ethyl ether. (It is good practice to leave the first extraction in the original funnel, and carry out the two subsequent extractions in another funnel.)

Wash the combined ether extracts as follows: Wash twice by pouring 100-cc. portions of distilled water through the ether extract; wash once by pouring 25 cc. of 0.25 *N* potassium hydroxide through the ether extract; wash twice by gently shaking with 30-cc. portions of distilled water.

Filter the washed ether extract into a 250-cc. Erlenmeyer flask. Evaporate the ether on a steam bath, under an atmosphere of carbon dioxide. As soon as the ether has evaporated, cool the flask quickly and dissolve the unsaponifiable residue in cyclohexane.

Make the solution up to 100 cc., shake thoroughly, and filter. Store the sample in the dark, at about 4° to 10° C. (40° to 50° F.), until ready to complete the determination spectrophotometrically. The determination should be completed on the same day that the extraction is made.

Determine the vitamin A content of the unsaponifiable solution by means of the spectrophotometer, using pure cyclohexane as a control and 1-cm. absorption cells.

Calculation of Instrument Factor.

$$E^{1\%}_{1\text{ cm.}}\text{ value} = \frac{\text{match point at 3280 Å.}}{\%\text{ concentration (on basis of original reference oil)}}$$

$$\text{Instrument factor} = \frac{\text{U. S. P. XI vitamin A units per gram of reference oil}}{E^{1\%}_{1\text{ cm.}}\text{ value}}$$

DISCUSSION. In order to establish the fact that the ultraviolet irradiation in the above method actually destroys the biological activity of the vitamin A and carotene in the unsaponifiable extract, the following test was made:

The solvent was vacuum-distilled from a solution of butterfat unsaponifiable which had been irradiated according to the above procedure; the irradiated unsaponifiable material thus recovered was then dissolved in hydrogenated cottonseed oil in an amount equivalent to the unsaponifiable content of the original butterfat. This sample of hydrogenated cottonseed oil, containing the irradiated butterfat unsaponifiable, was submitted to a biological laboratory for determination of its biological activity by the U. S. P. XI method.

The result of this assay, given in Table II, shows that the ultraviolet irradiation of the unsaponifiable material from butterfat actually destroys its biological vitamin A activity. Hence, the use of the irradiated control in the above method may be considered to be justified.

Stability of Extracted Carotene and Vitamin A. In order further to justify the conclusion drawn from Table II, it was necessary to demonstrate that (nonirradiated) carotene and vitamin A, extracted from butterfat by the above method and dissolved in hydrogenated cottonseed oil, would be stable in hydrogenated cottonseed oil for several weeks. At the same time, it was convenient to obtain a confirmation of the method by a biological assay.

A sample of butterfat was analyzed spectrophotometrically by the above method. The solvent was then vacuum-distilled from the nonirradiated sample, and the unsaponifiable material thus recovered was dissolved in hydrogenated cottonseed oil in an amount equivalent to the unsaponifiable content of the original butterfat. This hydrogenated cottonseed oil, containing the above butterfat unsaponifiable, was then periodically analyzed spectrophotometrically over a period of 11 weeks. During this 11-week interval, a portion of the same sample (of hydrogenated cottonseed oil containing the nonirradiated butterfat unsaponifiable) was sent to a biological laboratory for determination of its vitamin A content by the U. S. P. XI method.

TABLE II. BIOLOGICAL ASSAY OF IRRADIATED BUTTERFAT UNSAPONIFIABLE DISSOLVED IN HYDROGENATED COTTONSEED OIL

[Sample: hydrogenated cottonseed oil containing irradiated unsaponifiable from butterfat, in amount equal to unsaponifiable content of original butterfat. Biological (U. S. P. XI) method]

Level Assayed, U. S. P. Units Vitamin A per Pound	Daily Dose		Average Gain in Weight		U. S. P. Units Vitamin A per Pound
	U. S. P. reference oil	Sample	On reference oil	On sample	
	Mg.	*Mg.*	*Grams*		
Determine if sample has any vitamin A activity	0.5	500	27.8	Animals died after 11–20 days	Practically nil

TABLE III. STABILITY OF VITAMIN CONTENT OF BUTTER FAT UNSAPONIFIABLE DISSOLVED IN HYDROGENATED COTTONSEED OIL

Sample	Spectrophotometric Determination No.	Match Point At 4600 Å.	Match Point At 3280 Å.	Carotene[a]	Vitamin A[a]	Total[a]
Butterfat, unsaponifiable in cyclohexane solution	1 (12– 1–39)	0.85	0.95	5700	15,900	21,600
	2 (12–12–39)	0.85	0.95	5700	15,900	21,600
					Av.	21,600
		40 Per Cent Solution				
Hydrogenated cottonseed oil containing nonirradiated unsaponifiable from above butterfat in an amount equal to the unsaponifiable in original butterfat	1 (12–12–39)	0.65	0.80	5500	16,600	22,100
	2 (12–26–39)	0.63	0.75	5300	15,800	21,100
	3 (2–1–40)	0.65	0.80	5500	16,600	22,100
					Av.	21,800

Biological (U. S. P. XI) Method

Sample	Level Assayed, U. S. P. Units Vitamin A per Pound	Daily Dose U. S. P. reference oil	Daily Dose Sample	Average Gain in Weight On reference oil	Average Gain in Weight On sample	U. S. P. Units Vitamin A per Pound
		Mg.	*Mg.*	*Grams*	*Grams*	
Hydrogenated cottonseed oil containing nonirradiated unsaponifiable from above butterfat in an amount equal to the unsaponifiable in original butterfat	19,000	0.5	35.8	27.8	27.1	19,000
	24,000	0.5	28.3	27.8	19.4	

[a] As U. S. P. units of vitamin A per pound of butterfat, rounded off to nearest 100 units.

The results presented in Table III show that carotene and vitamin A, extracted from butterfat and dissolved in hydrogenated cottonseed oil, are stable in the hydrogenated cottonseed oil for at least the 11 weeks during which the sample was under test (hence the conclusion drawn from Table II is still further justified). These results also show that the method gives results which are in reasonable agreement with the U. S. P. XI method (on this one sample).

Duplication of Results. The above method has been used for the determination of the total vitamin A content of many samples of dairy butter, produced over a period of more than a year, and has been found to give duplicate results within very good agreement. Typical results are tabulated in Table IV for the purpose of showing the limits of variability which may be expected from this method. From these results it may be concluded that duplicate results obtained by the above method, applied to butters of widely varying vitamin content, agree to within less than ±6 per cent.

Further Confirmation of Results by Biological Assays. In order to determine the agreement between the above spectrophotometric method and the biological method for the determination of vitamin A in dairy butters of widely varying vitamin content, samples 1, 2, 4, and 5 listed in Table IV were sent to a biological laboratory for assay by the U. S. P. XI method. These samples were biologically assayed on the separated butterfat. The results of this comparison (by the two methods) are listed in Table V. Bearing in mind the known variation in results obtainable by biological methods, the data given in Table V may be considered to indicate that results obtained by the spectrophotometric method are in reasonable agreement with those obtained by the U. S. P. XI method.

Effect of Added Coloring Agents. The method is entirely satisfactory for butter colored with annatto, since the caustic treatment and subsequent washings adequately remove the annatto coloring materials. However, azo dyes seriously interfere, and the method is not satisfactory for butter colored with F. D. & C. yellows 3 and 4, formerly known as AB and OB, respectively. These dyes are not removed by the saponification process, and are affected by the ultraviolet light irradiation. Hence samples which show, by chemical test, the presence of these dyes have necessarily been discarded without attempting to determine their vitamin A content by the above method.

Summary

A spectrophotometric method for the determination of the total vitamin A content, including carotene, in dairy butter has been developed. This method is based on the destruction of both the carotene and vitamin A in a portion of the unsaponifiable extract by ultraviolet light irradiation, and the use of this irradiated portion as a control for the simultaneous determination of both carotene and vitamin A by means of the spectrophotometer. The method has been shown to yield results that are in satisfactory agreement within themselves and in reasonable agreement with the U. S. P. XI method.

TABLE IV. LIMITS OF VARIABILITY ON RESULTS FOR A GIVEN SAMPLE

Sample No.	Spectrophotometric Determination No.	Match Point (*vs.* Irradiated Control) At 4600 Å.	Match Point (*vs.* Irradiated Control) At 3280 Å.	Carotene[a]	Vitamin A[a]	Total[a]
1	1	0.87	0.96	5800	16,100	21,900
	2	0.88	0.88	5900	14,400	20,300
	3	0.95	1.0	6300	16,500	22,800
2	1	1.20	1.10	8000	17,900	25,900
	2	1.25	1.05	8400	16,700	25,100
	3	1.25	1.10	8400	17,700	26,100
3	1	0.18	0.30	1200	5,200	6,400
	2	0.18	0.28	1200	4,800	6,000
	3	0.18	0.28	1200	4,800	6,000
4	1	0.22	0.60	1500	11,100	12,600
	2	0.22	0.55	1500	10,100	11,600
	3	0.20	0.55	1300	10,100	11,400
5	1	0.12	0.25	800	4,500	5,300
	2	0.12	0.25	800	4,500	5,300

[a] As U. S. P. units of vitamin A per pound of butterfat, rounded off to nearest 100 units.

TABLE V. COMPARISON BETWEEN SPECTROPHOTOMETRIC AND BIOLOGICAL METHODS

Sample No.	U. S. P. Units Vitamin A per Pound of Butterfat, Range by Spectrophotometric Method	Biological (U. S. P. XI) Method: Level assayed, U. S. P. units vitamin A per pound of butterfat	Daily Dose U. S. P. reference oil	Daily Dose Sample	Average Gain in Weight On reference oil	Average Gain in Weight On sample	U. S. P. units vitamin A per pound of butterfat
			Mg.	*Mg.*	*Grams*	*Grams*	
1	20,300–22,800	21,000	0.5	32.43	24.9	20.2	A little less than 21,000
2	25,100–26,100	25,000	0.5	27.24	24.9	28.0	A little more than 25,000
4	11,400–12,600	10,000	0.67	90.9	42.0	37.1	A little less than 10,000
		12,000	0.67	75.5	43.6	30.3	
5	5,300	4,000	0.66	227.0	32.4	41.4	A little more than 6000
		6,000	0.66	151.3	32.4	34.4	

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Test Formulas for Reclaimed Rubber

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Compounded test formulas are more desirable for testing reclaimed rubber than the reclaim-sulfur mix, because the test results are more consistent and more indicative of the quality of the reclaimed rubber.

Six types of acceleration which might be used in test formulas were investigated, and the combination of dibenzothiazyldimethylthiolurea and diphenylguanidine was found to be satisfactory for most types of reclaimed rubber.

Several specific recommendations of compounded test formulas for reclaimed rubber are made. If the rubber and fillers are master-batched, the test requires no more time and labor than the reclaim-sulfur test.

IN 1934, Palmer (*3*) presented a definite program for the testing of reclaimed rubber in which it was stated that physical tests in a test recipe containing reclaimed rubber were of definite value if they were known to have a direct and accurate interpretation in reference to the tests on the finished product. This procedure was made secondary to an actual factory run with the reclaim mixed in typical compounds. In the latter case, of course, the tests may be run by the consumer of the reclaimed rubber, whereas in the former case they may be run by the consumer or the manufacturer. It is the desire of the manufacturer to test his product in a manner which properly interprets it for the consumer. For this purpose, test formulas have been coming into fairly general use, and inasmuch as there are innumerable formulas which may be used, it is most desirable and essential to have some uniformity for the sake of universal comparison and simplicity of operation. It is the purpose of this paper to point out the greater uniformity of results obtained with compounded test formulas and to make definite recommendations of several such formulas.

The ideal test formula should not only measure the uniformity of reclaimed rubber from lot to lot, but should also predict to some extent its performance when used in the consumers' products. Although the reclaim-sulfur mix is still used by many for this purpose, it is recognized by most of the reclaim manufacturers and many of the larger consumers that this formula gives erratic and undependable results. The reclaim-sulfur mix contains 100 parts of reclaimed rubber and 5 parts of sulfur, and is generally cured from 15 to 35 minutes at 141.7° C. (287° F.). Winkelmann (*7*), Stafford (*6*), Hurleston (*2*), and Palmer (*3*, *4*) have either mentioned or protested the lack of correlation of the reclaim-sulfur test with the compounding value of the reclaim and have pointed out the shortcomings of this test. It has been shown (*3*) that the reclaim-sulfur test is accurate at best to only 10 per cent and that even the best results are not a criterion of the properties of the reclaim in the recipe in which it is to be used. Maximum variations of from 12 to 19.5 per cent of the mean in the tensile strength and from 10 to 11 per cent in elongation in the reclaim-sulfur mix were reported in 1940 by Palmer and Crossley (*5*).

In contrast to the above inconsistencies, the variation involved in the use of a compounded test recipe is definitely less, as shown in Table I. These tests were run in test formulas IV and V (Table VII). Equal parts cut from three slabs of whole tire reclaim A were thoroughly blended on the mill and from this blend six mixes of each formula were made, two

Table I. Variation in Physical Tests of Test Formulas

		Formula IV, Cure 6 Minutes at 158° C.		Formula V, Cure 12 Minutes at 148° C.	
Mix No.	Slab No.	Elongation %	Tensile strength *Kg./sq. cm.*	Elongation %	Tensile strength *Kg./sq. cm.*
1	1	415	90.7	585	170.8
	2	410	88.6	600	172.2
2	1	410	91.7	590	171.5
	2	390	87.2	605	172.9
3	1	380	88.2	605	181.7
	2	375	87.9	600	175.1
4	1	395	91.0	585	175.8
	2	380	87.5	580	175.8
5	1	380	87.5	585	177.9
	2	395	92.4	575	176.1
6	1	410	91.0	585	175.4
	2	390	88.6	585	178.9
Average		394	89.4	590	175.3
Maximum variation		40	5.2	30	10.9
Per cent of average		10.1	5.9	5.1	6.2

Table II. Analyses of Reclaims

	A	B	C
Type	Whole tire	Whole tire	Tube
Color	Black	Black	Neutral
Manufacturing process	All by alkali digestion		
Specific gravity, grams per cc.	1.15	1.15	1.25
Chemical tests			
Acetone extract	8.80	10.80	7.50
Ash	16.50	15.70	32.00
Total sulfur	2.10	1.90	2.10
Carbon black	12.70	14.50	0.70
Alkalinity (as NaOH)	0.20	0.50	0.20
Rubber content (by difference)	59.90	57.10	57.70

TABLE III. FORMULAS AND PHYSICAL PROPERTIES

Type	I Mechanical molded goods	II Tire carcass	III Tire carcass
Test Formulas			
Smoked sheets	10.00	...	31.62
Brown crepe	...	17.00	...
Reclaimed rubber	50.00	65.00	50.00
Zinc oxide	2.00	1.50	1.25
Natural whiting	15.40	9.62	12.00
Clay	20.00	2.00	...
Stearic acid	0.60	0.50	0.75
Pine tar	...	1.50	2.00
Sulfur	1.40	2.50	2.00
Diphenylguanidine	0.60	...	...
Mercaptobenzothiazole	...	0.38	0.38
	100.00	100.00	100.00
Range of Physical Properties			
Time of cure, minutes	6	25	25
Temperature of cure, ° C.	158	141.7	141.7
Elongation, per cent	385–435	485–535	600–650
Tensile strength, kg. per sq. cm.	70–100	105–135	140–170
Shore Type A hardness	67–72	52–57	45–50

TABLE IV. RECLAIM-SULFUR MIX *vs.* TEST FORMULAS

Reclaim	Formula	Cure		No. of Tests	Tensile Strength				Elongation			
					Max.	Min.	Av.	Variation	Max.	Min.	Av.	Variation
		Min.	*° C.*		*Kg./sq. cm.*			*% of average*	*%*	*%*	*%*	*% of average*
A	Reclaim-sulfur	25	141.7	36	56.2	39.7	45.7	29	405	300	355	30
B	Reclaim-sulfur	25	141.7	57	91.4	56.2	69.6	51	470	360	405	27
C	Reclaim-sulfur	25	141.7	30	90.3	53.8	66.8	55	595	460	520	26
A	I	6	158	28	98.1	82.3	86.8	18	480	380	425	24
B	II	25	141.7	54	131.8	107.2	114.9	21	530	435	495	19
C	I	6	158	34	103.0	85.4	90.7	19	510	430	460	17

color, all of which are manufactured by the alkali digestion process.

Over a period of 9 months, a considerable number of lots of each reclaim were tested, using a blend of five random samples from each lot for each test. Each blend was simultaneously tested in the reclaim-sulfur mix and in test formulas I or II, as indicated in Table IV. There is up to three times as much variation in tensile strength and up to one and one-half times as much variation in elongation from test to test in the reclaim-sulfur mixes as in the corresponding test formula mixes.

In Table V a comparison has been made of the elongations and tensile strengths of the reclaim samples displaying the greatest variation in reclaim-sulfur tensile strength, with the corresponding tests obtained when the same sample of reclaim is tested in test formulas I, II, and III. The variations in reclaim-sulfur tensile strengths and elongations are large and are not reflected in the results obtained from the test formulas. Consequently, the reclaim-sulfur test cannot be used as an indication of reclaim quality.

These are only a few of the many instances of the unreliability of the reclaim-sulfur test which have been experienced. In most cases the variation in reclaim-sulfur tensile strength is not reflected in the final compound. However, in certain types of compounds where reclaim is the main ingredient, variations in tensile strength of reclaim in the reclaim-sulfur test may carry over to the compound, showing a trend, but the differences are not proportional to those observed in the reclaim-sulfur test.

Throughout this paper, tensile strength and elongation have been considered the criteria of quality by which a compound is judged. In some cases these properties are not entirely indicative of the performance of the ultimate product, and it frequently becomes desirable to obtain tests which more nearly duplicate the service conditions of the final product. For example, such tests may be abrasion and flexing resistance, cold and hot compression set, resistance to heat build-up, resistance to artificial aging, and many others, any of which may be obtained from test formulas, and which serve as definite indications of what may be expected in service, whereas such tests run on the reclaim-sulfur mix would have very little meaning. The authors do not mean to imply that the use of test formulas is the panacea to cure all testing ills, but it is believed that by their use the manufacturer and consumer of

slabs from each mix were cured, and three strips from each slab were tested. All tests were made in accordance with the standard practice as recommended by the A. S. T. M. (*1*) except that the compounds were aged only 4 hours between mixing and curing. The maximum variations in tensile strength from test to test for test formulas IV and V are 5.9 and 6.2 per cent of the mean, respectively, as compared to the figures of 10, 12, and 19.5 per cent previously referred to for the reclaim-sulfur test. The maximum variations in elongation from test to test for formulas IV and V are 10.1 and 5.1 per cent of the mean, respectively, as compared to 10 and 11 per cent previously reported for the reclaim-sulfur test.

Throughout this paper the term "maximum variation from test to test" is used to describe the variation in physical properties of reclaimed rubber, rather than the more mathematically precise "root mean square error" or "standard deviation", because it is believed that the actual extent to which any one test might differ from any other is of more interest than a mathematical expression of the probable error of any one test. From the point of view of control testing, it is the extreme variations from test to test in the reclaim-sulfur mix which have been most confusing, and these extremes are not emphasized if the variations are expressed as "standard deviations".

As further evidence of the unreliability of the reclaim-sulfur test, a comparison was made of three typical reclaims in the reclaim-sulfur mix and in test formulas I, II, and III (Table III). The characteristic analyses of the reclaims used are given in Table II. A and B are whole-tire reclaims, while C is a tube reclaim of neutral

TABLE V. RECLAIM-SULFUR MIX *vs.* TEST FORMULAS

Reclaim	Formula	Cure		Tensile Strength			Elongation		
				Sample having highest tensile	Sample having lowest tensile	Difference	Sample having highest tensile	Sample having lowest tensile	Difference
		Min.	*° C.*	*Kg./sq. cm.*			*%*	*%*	*%*
A	Reclaim-sulfur	25	141.7	56.2	39.7	16.5	405	320	85
	I	6	158	92.5	92.1	0.4	425	435	10
B	Reclaim-sulfur	25	141.7	91.4	56.2	35.2	440	370	70
	II	25	141.7	128.7	115.2	13.5	480	505	25
B	Reclaim-sulfur	25	141.7	82.6	52.7	29.9	685	440	225
	III	25	141.7	163.1	163.8	0.7	645	605	40
C	Reclaim-sulfur	25	141.7	90.3	53.8	36.5	595	460	135
	I	6	158	92.8	91.8	1.0	460	460	0

Table VI. Acceleration for Test Formula

Type of Acceleration	Number of Different Reclaims Tested	Number of Reclaims Reaching Optimum Properties, at 158° C.		
		4 Min.	*6 Min.*	*8 Min.*
Diphenylguanidine	13	None	5	8
Dibenzothiazyldimethylthiolurea	6	2	2	2
Zinc salt of mercaptobenzothiazole and di-*o*-tolylguanidine	4	None (All tensile strengths low)	4	None
Dibenzothiazyldimethylthiolurea and diphenylguanidine	13	2	10	1
Zinc salt of mercaptobenzothiazole	4	None (All tensile strengths low)	2	2
Benzothiazyl disulfide	7	3	2	2

reclaimed rubber may more nearly approach the perfect test, which is, of course, an actual production trial in the consumer's factory, with service tests on the resultant product.

Accelerator Combinations

It has been suggested (*3*, *4*, *5*) that the most desirable test formula to use is one typical of that in which the reclaim will be used. For a routine production control test, however, this is not practical, as it would involve a great number of formulas and considerable laboratory work. Accordingly, it seems desirable to standardize on one or two simple formulas of general nature, which could be used to control the uniformity of all reclaims. Formula I (Table III) answers this requirement, except that reclaims having different rates of cure, when tested in this formula, fail to attain their optimum properties in the same curing time.

For example, reclaim highly alkaline in reaction might reach its optimum cure in 5 to 6 minutes, while a neutral reclaim might require 8 to 10 minutes. Thus, it would be necessary to have a special cure for each type of reclaim. It seems desirable from an efficiency standpoint to standardize on one cure for all reclaims if possible; accordingly, a study was made of a number of different accelerator combinations in an effort to find one which would be flexible enough to meet this condition. These results are shown in Table VI. The reclaims used in this work were typical of all the different types commonly produced. While no one accelerator combination was found to be completely satisfactory, the combination of dibenzothiazyldimethylthiolurea and diphenylguanidine appeared to be the best, since it showed 10 optimum tensiles at 6 minutes out of the 13 reclaims tested.

As only a few of the more common accelerator combinations were investigated, there are doubtless many others which would prove equally satisfactory.

Recommended Formulas

In Table VII are listed three test formulas which are recommended for testing reclaimed rubber. Formulas IV and V employ the dibenzothiazyldimethylthiolurea-diphenylguanidine accelerator combination, and are suggested for routine control testing purposes to replace the reclaim-sulfur test. For simplification in handling, and for time and labor saving, the rubber and fillers in these formulas can be master-batched so that it is only necessary to weigh reclaim and master batch for the final mix. In some cases, it might be desirable also to omit the sulfur from the master batch and add it to the final mix, in which case, three weighings would be required, which compares to two weighings for the reclaim-sulfur mix. If the master batch method is used, the final mix can be made in 5 to 6 minutes, which compares favorably to the time required to mix a reclaim-sulfur batch. The curing time for the test formulas (6 to 12 minutes) is less than for the reclaim-sulfur mix (generally 15 to 35 minutes). The point to be emphasized here is that in replacing the reclaim-sulfur test with a compounded test formula, there need be no increase in the time and labor of testing.

Formula III (Table III) and formula VI (Table VII) are suggested as typical of compounds to be used in testing reclaims for use in carcass or tread compounds. Various tests may be obtained, such as stress, elongation, tensile strength, hardness, artificial aging, abrasion and flexing resistance, and any others which aid in predicting the performance of the reclaim in service.

At the request of the consumer, the manufacturer can and does test reclaimed rubber in a specific test formula which the consumer may submit for this purpose. On the other hand, the consumer may agree to be guided by the results of tests from a test formula suggested by the manufacturer. It is hoped that the suggestion of formulas IV and V particularly, as well as formulas III and VI, will aid in the development of this more practical method of testing reclaimed rubber.

Table VII. Formulas and Physical Properties

Type	IV	V	VI
	General purpose molded goods		Tire tread
Test Formulas			
Smoked sheets	10.00	36.00	36.00
Reclaimed rubber	50.00	50.00	40.00
Zinc oxide	2.00	3.30	2.50
Clay	20.00	4.00	..
Whiting	15.30	2.60	..
Carbon black	..	..	17.70
Stearic acid	1.00	1.30	1.70
Sulfur	1.40	2.31	1.75
Mercaptobenzothiazole	..	..	0.35
Diphenylguanidine	0.10	0.16	..
Dibenzothiazyldimethylthiolurea	0.20	0.33	..
	100.00	100.00	100.00
Range of Physical Properties			
Time of cure, minutes	6	12	35
Temperature of cure, ° C.	158	148	134.5
Elongation, per cent	375–425	575–625	550–600
Tensile strength, kg. per sq. cm.	70–100	160–190	195–225
Shore Type A hardness	70–75	48–53	65–70

Conclusions

The maximum variation from test to test obtained when testing a sample of reclaimed rubber in compounded test formulas is approximately half that experienced when the reclaim-sulfur test is used.

When tested with sulfur alone, reclaimed rubber may show a widely varying tensile strength and elongation from lot to lot, whereas much less variation in results is observed if the reclaimed rubber is tested in a compounded formula. For this reason, and also because the results obtained are more indicative of final performance, test formulas of a general nature are preferable to the reclaim-sulfur test.

When test formulas are used in preference to the reclaim-sulfur mix, there is no increase in the work of testing.

A combination of dibenzothiazyldimethylthiolurea and diphenylguanidine gives very satisfactory results for most types of reclaim.

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Chemical Constituents of Cottonseed Hulls

A Partial Separation by Nonchemical Methods

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Crushed cottonseed hulls, extracted in alcohol-benzene, were ground for a suitable time in a ball mill and the less fibrous portion was separated by sieves and by sedimentation from water into coarse and fine fractions. These were analyzed by standard methods for pentosan, uronic anhydride, and "lignin" (no assumptions were made concerning the chemical nature of the hull constituent responsible for the analytical data). The original hulls contained pentosan, uronic anhydride, and "lignin" to the extent of 27, 6.5, and 25 per cent; the corresponding figures were approximately 3, 9, and 50 per cent for the finest fraction and 40, 6, and 24 per cent, for coarser ones. Thus, unusually rich concentrates of either pentosan or "lignin" are potentially available to industry, although large-scale uses for them have yet to be discovered. The possibility that some other species of plant material, particularly other types of hulls, would respond to the new analytical technique was not investigated.

The detailed results on cottonseed hulls were in agreement with the hypothesis that the pentosan and the "lignin" were present as separate chemical complexes, both of which included uronic anhydride. Klason "lignin" prepared from the hulls contained no methoxyl groups and differed sharply, in this respect at least, from wood lignins.

A MAJOR difficulty in studying the chemistry of plant tissue is caused by the sensitiveness of the components to chemical change during isolation, usually by extraction of the plant material with acid or alkaline solutions or by hydroxylic organic solvents. Lignin, for example, has never been separated in substantial amount in a native condition and the question as to whether it exists as a chemical individual in the plant or is combined with other constituents is still undecided. While studying the hull of the cottonseed, routine estimations led to the conclusion that the dust from the hulls differed markedly in composition from coarser fragments. This foreshadowed a new method of separation which could be conducted without the possibility of chemical change, although the efficiency would probably vary with the botanical structure and chemical composition of the various species of plants. The present study describes the nature and extent of such nonchemical separations of cottonseed hull constituents. Published work on these hulls has been restricted to analytical data (*10*), attempts to find economic uses for them (*11*), and detailed investigations of hemicellulose fractions (*3*, *4*).

The hulls, which had not been treated in any way with chemicals, consisted of oily, hard, cup-shaped brown fragments covered with short fiber. They were exhaustively extracted with hot alcohol-benzene and dried in the air. A qualitative test for nitrogen was negative.

Methods of Analysis

All "lignin" (material insoluble in sulfuric acid), pentosan, and uronic anhydride estimations were made on samples ground to pass completely through a 100-mesh screen, as the impermeable nature of the hulls (*18*) caused very erratic results with particles of larger size. Concordant duplicates were obtained throughout and the data were corrected for moisture, determined by heating separate samples at 105° C.

Samples of weight sufficient to give 0.1 to 0.5 gram of residue were used in the "lignin" determination. They were dispersed in 2 cc. of alcohol, 25 cc. of cold 72 per cent sulfuric acid were added, and, after keeping the mixture at 20° C. for 24 hours, the estimation was completed by a standard method (*15*). The presence of the alcohol prevented caking of the finely divided material on addition of the acid and did not affect the result seriously. Found: no alcohol, "lignin" 25.57, 25.25; with alcohol, 25.27, 25.00 per cent. Klason "lignin" prepared in this way had the composition: carbon, 60.7; hydrogen, 5.23; sulfur, 0; methoxyl, 0 per cent (corrected for 0.4 per cent of ash).

No correction was made in the uronic anhydride analyses for the small amount of carbon dioxide evolved from hexoses (*19*). A sample weight of about 2 grams in 100 cc. of 12 per cent hydrochloric acid was heated in a bath kept at 135° for 5 hours in all cases. Whenever possible, the customary estimation for pentosan (*1*) was corrected for uronic anhydrides by means of a relationship derived from artificial mixtures of xylose and glucuronic acid (euxanthic acid) (*5*). The validity of this proceeding was doubtful, but no more appropriate correction was found.

Cross and Bevan cellulose was prepared generally as described by Doree (*6*), chlorine being passed for a few minutes through an aqueous suspension of the finely divided hulls to minimize local heating. Three chlorinations, alternated with hot sodium sulfite extractions, left a white powder containing 0.4 to 3.5 per cent of "lignin".

An attempted isolation of holocellulose (*14*) from finely ground hulls gave a product containing 17 per cent of "lignin" after four chlorinations and extractions with alcohol-pyridine at room temperature. Still less success was obtained with unground samples.

Table I. Milling Conditions and Composition of Hull Fractions

Milling	Fiber Yield	Nonfibrous Fractions				Per Cent of Total	
		Screen	Yield	"Lignin"	Pentosan[a]	'Lignin"	Pentosan[a]
Hours	%	*Mesh*	%	%	%		
12	0	<100	100	25.1	32[b]	100	100
6	11	>60	38	20	46	44	77
		60–100	16	21	44	..	..
		<100	35	33	13	46	14
4	12	>60	43	22	45	46	72
		60–100	9	22	42	..	..
		<100	29	30	19	35	17
2	31	>60	49	23	45	45	69
		60–100	0	..	..	..	..
		<100	20	36	10	29	6

[a] Not corrected for uronic anhydride.
[b] 27.3% after correction for uronic anhydride.

Milling Conditions and Screening Experiments. The extracted hulls plus attached fiber, 200 grams, were disintegrated in a rotating ball mill (3.785-liter, 1-gallon, capacity) three quarters full of pebbles, and the product was separated into fractions by shaking through sieves of different mesh. Much of the visible fibrous material survived the shorter periods of milling and formed mats upon the screens from which it was readily removed by hand. As appreciable quantities of adhering hull dust and fragments were entrapped in these mats, the yields of fibers quoted in Tables I and II are high.

Table II. Analysis of Hulls Milled for 2 Hours

Hull Fraction	Screen Analysis[a]	"Lignin"	Uronic Anhydride	Pentosan[b]	Per Cent of Total "Lignin"	Per Cent of Total Uronic anhydride	Per Cent of Total Pentosan[b]
	%	%	%	%			
A. Coarse fiber	32.2	24.0	6.14	24.9	30.8	30.7	29.4
B. Fine fiber	9.8	23.4	5.82	15.6	9.1	8.8	6.0
C. Hulls (fiber-free) >30-mesh	30.7	23.9	6.63	34.0	29.2	31.4	38.2
D. Hulls (fiber-free) 30- to 60-mesh	20.6	25.1	7.25	33.4	20.6	23.0	25.2
E. Hulls <60-mesh	6.8	39.7	8.20	8.1	10.8	8.6	2.0
	100.1				100.5	102.5	100.8
Original ground hulls[c]		25.1	6.48	27.3	100	100	100

[a] Based on air-dry weights.
[b] Corrected for uronic acid.
[c] 2.34 kg.

Twelve hours of milling resulted in a powder, all of which passed through a 100-mesh screen. The "lignin" and pentosan contents of 25.1 and 27.3 per cent, respectively, were therefore the average for the original material These values were in general accord with published data (*2*, *9*, *10*, *20*). With 6 hours or less of grinding, nonfibrous fractions which failed to pass the 100-mesh screen were very similar in composition and were combined in the calculation of the over-all "lignin" and pentosan yields quoted in Table I. While 4 to 6 hours of grinding gave the maximum yield and concentration of pentosan in coarse fractions, less drastic comminution for 2 hours resulted in a fraction passing the 100-mesh screen and containing no less than 36 per cent of "lignin". The same short grinding period was adopted for a larger scale experiment in which the whole of the original material, including fiber mats from the coarser and finer screens, was finally obtained as fractions A to E of Table II. The three right-hand columns of Table II give the amounts of "lignin", uronic anhydride, and pentosan contained in each fraction as a percentage of the total amount. In each case the recovery approximated 100 per cent with an accuracy which probably owed something to a fortuitous cancellation of experimental errors. The fine powder, E, contained 10.8 per cent of all the "lignin" in a state of 39.7 per cent purity. If "lignin" associated with the fibrous fractions was not considered, the above yield became 17.9 per cent. This was not necessarily the maximum possible, because a 2-hour regrinding of the coarse hull fraction, C, with 23.9 per cent of "lignin", reduced one fifth of it to fines with 31.9 per cent. The amount and composition of the various fractions naturally depended greatly on the milling conditions.

Separation by Air Blast. About 50 grams of the powdered, fiber-free hulls were placed in a vertical glass column 90 cm. (3 feet) high and about 6.25 cm. (2.5 inches) in diameter. The lower end was fitted with a 5-cm. (2-inch) filter funnel held in place by a rubber stopper and with the dead space between funnel and column packed with cotton. Compressed air, introduced through the funnel stem, continuously removed the center portion of the charge and was regulated to blow the largest particles one half to two thirds of the way up the column. These fell back to the center of the funnel, while the finer particles issuing from the top of the column as a smoke were led through a tube 2.5 cm. (1 inch) in diameter to a series of water traps. Four were sufficient if the smoke was directed against the surface of the water. In the authors' experiments, 15 to 20 per cent of the original charge was collected and contained 34.4 per cent of "lignin" and 18.1 per cent of pentosan (uncorrected). The efficiency of the process naturally varied greatly with the experimental conditions and there seems to be no good reason why suitable equipment would not recover the smoke without wetting it.

Separation by Sedimentation in Water. The glass column was nearly filled with distilled water and 100 to 200 grams of powdered, fiber-free hull fractions approximating in composition 40 per cent "lignin" and 8 per cent pentosan were added. A few seconds of agitation with the air blast gave a uniform suspension which settled slowly to form a deposit consisting of visibly distinct, horizontal layers. A tedious filtration of the supernatant suspension through these layers increased their definition and made it easy to separate them mechanically for analysis. In most cases, however, the supernatant liquor was decanted. Material still in suspension was recovered in a separate filtration, steeped in acetone, and dried *in vacuo*. The observed "lignin" contents were 41.0, 50.8, and 53 per cent after sedimentation for 2, 6, and 12 hours, respectively. An accumulation of about 100 grams averaging 50 per cent "lignin" was fractionated by resuspension in water for 6 hours. Found, "lignin", 51.2, 50.8, 50.0; pentosan (uncorrected), 6.4, 5.4, and 6.2 per cent for the second, third, and the deposit from the third suspension, respectively. Resolution by means of fractional sedimentation from water had therefore reached its limit. A portion of the original, alcohol-benzene–extracted hulls, ground in the ball mill in presence of water, also gave a similar 50 per cent "lignin" fraction. This experiment eliminated the possibility that the concentrate originated through local heating in the dry milling which was usually employed to disintegrate the hulls.

Examination of Suspensoid Fraction

A very rough calculation from the rate of settling in water and from Stokes' law gave an average particle radius of the order of 50 microns for the suspensoid (50 per cent "lignin") fraction in its original state. It consisted of a brown, moist cake which formed a very hard mass when dried directly from water and a very fine powder when the water was removed by solvent exchange with acetone. The fraction was a mixture whose composition varied somewhat in different preparations. The analyses (Table III) were typical and it will be noted that about 15 per cent of the material escaped estimation. The cause of this discrepancy requires further investigation, although it is possible that the Cross and Bevan determination failed to include all carbohydrate not already estimated as pentosan or uronic anhydride.

A 0.215-gram sample was tested for pectic substances by heating it on the steam bath for 16 hours with 50 cc. of aqueous 0.5 per cent ammonium oxalate at pH 5. The almost colorless filtrate was discarded and the residue, washed with water and dried at 105°, weighed 0.198 gram or 92 per cent of the original. Another sample lost 2 per cent by weight, including some ash, when extracted with glacial acetic acid at room temperature.

Table III. Analysis of Suspensoid Fraction

	%
C[a]	50 ± 1[b]
H[a]	6.2 ± 0.3
OCH_3[a]	0.0
"Lignin"[a]	50.5
Pentosan[c]	3
Uronic anhydride	9.1
Cross and Bevan cellulose[d]	15.3
Ash	6.5
Total	84.4

[a] Corrected for ash.
[b] Individual carbon analyses were 46.3, 47.6, 50.0, 49.1 (3.6% ash), and 48.9, 47.7, 50.2, 49.2 (2.0% ash).
[c] Corrected for uronic anhydride. Uncorrected, 6.4%.
[d] Corrected for ash, pentosan, and "lignin".

Discussion

Cottonseed hulls, digested with cold 72 per cent sulfuric acid, gave a methoxyl-free residue which is called "lignin" to emphasize its undetermined relationship to the methoxyl-containing lignins similarly prepared from woods. This lack

Table IV. Rations of "Lignin" and Pentosan to Uronic Anhydride

Fraction	% "Lignin" / % Uronic Anhydride	% Pentosan / % Uronic Anhydride	Moles Pentosan / Moles Uronic Anhydride Corrected
A. Coarse fiber	3.9	4.1	18.0
B. Fine fiber	4.0	2.7	15.3
C. Hulls >30-mesh	3.6	5.1	19.2
D. Hulls 30- to 60-mesh	3.5	4.6	16.1
E. Hulls <60-mesh	4.8	1.0	9.9
Suspensoid (50% "lignin")	5.6	0.3	..
Original hulls plus fiber	3.9	4.2	18.3

of the methoxyl group, in conjunction with the failure to account for 16 per cent of the fine hull fraction (Table III), suggested that the 50 per cent yield of residue obtained in this case was an artifact produced by the action of strong acid upon sensitive hull constituents. The latter were probably not tannins (*20*) because these are usually extracted from plant materials by water or acetone (*16*), and repeated treatment of the very finely divided, suspensoid hull fraction with these solvents failed to diminish the yield of "lignin".

The pentosan, uronic anhydride, Cross and Bevan cellulose (Table III), and also the substances extracted by a hot pectic solvent (8 per cent), were insufficient in amount to account for the 50 per cent residue obtained with sulfuric acid. Thus, if the latter originated with sensitive carbohydrates (*7*), these were not detected by the usual carbohydrate analyses. Although individual microcombustions were somewhat discordant, perhaps owing to sampling errors, the average carbon content of the fine hull fraction was about 50 per cent. Assuming 44 to 45 per cent carbon for the 30 per cent of the hulls which analyzed as carbohydrate, the remaining 70 per cent (on an ash-free basis) had a calculated carbon content of 51 to 52 per cent. This was sufficiently high to eliminate sensitive carbohydrates as precursors of the Klason residue. Whatever the chemical nature of these precursors might be, they reacted like ordinary lignin in the Cross and Bevan determination and gave a similar color change during the operation. They were doubtless related to the "Body X" extracted from the hulls by alkali (*4*) and their zero methoxyl content placed the cottonseed in an extreme position with respect to hulls of other species, which sometimes give Klason residues with low methoxyl values (*8*). Young cereal shoots are in the same category and it has been suggested (*13*) that in them the lignin structure is first laid down and then methylated at a later stage. Perhaps the latter step is partially or completely inhibited in the metabolism of hulls. But speculation concerning the nature of the residues obtained from hulls by strong acid is not germane to the following discussion. The reasoning is based entirely on data from standardized estimations and is independent of assumptions concerning the structure of the hull constituents which respond to the conventional procedures for determining lignin, pentosan, and uronic anhydride.

Consideration of Tables I, II, and III shows very clearly that purely physical methods, based on particle size and density, separated ground cottonseed hulls into fractions containing about 40 to 3 per cent of pentosan, 20 to 50 per cent of "lignin", and 5.8 to 9.1 per cent of uronic anhydride. The same technique, when applied to groundwood of spruce and maple, produced analytical fluctuations of only a few per cent. Other workers with groundwood had a similar experience and found that both pentosan and lignin accumulated very slightly in the finest fractions, which amounted to 42 to 69 per cent of the entire material (*12*, *17*). The fine hull fraction, E, 6.8 per cent of the total, was rich in "lignin" and poor in pentosan (Table II). This contrast between hulls and groundwood may depend to an unknown degree upon the milling conditions and upon the particular species examined. Uronic anhydride accumulated with the "lignin" in the finer fractions of the hulls and the ratio between the two was much more constant for various sized particles than the pentosan–uronic anhydride ratio. This is illustrated in the second and third columns of Table IV, which were derived from the data of Tables II and III.

If the assumption is made that the uronic anhydride was in part chemically associated with pentosan and in part with "lignin", the ratio of the components in the latter complex could be taken as 1 to 5.6, since the suspensoid fraction (Table III) was almost pentosan-free. If this ratio was also true for the other fractions, it was possible in each case to calculate the molar ratio of pentosan to that anhydride not assumed to be associated with "lignin". The results (Table IV, column 3) varied between 9.9 and 19.2 to 1 and may be compared with the pentosan–uronic anhydride ratios of 10 and 16 to 1 found from estimations of hemicelluloses isolated from the hulls (*3*). Thus, although the above assumptions lack experimental proof, they are not in conflict with the data at hand.

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Correction. In the article on "Determination of Methionine in Certain Mixtures" [Ind. Eng. Chem., Anal. Ed., **12**, 723 (1940)] references (2), (3), (4), and (5) are all to the *Journal of Biological Chemistry*.

J. J. Kolb

Determination of the Proportion of *d*- and *l*-Isomers in Samples of Lactic Acid

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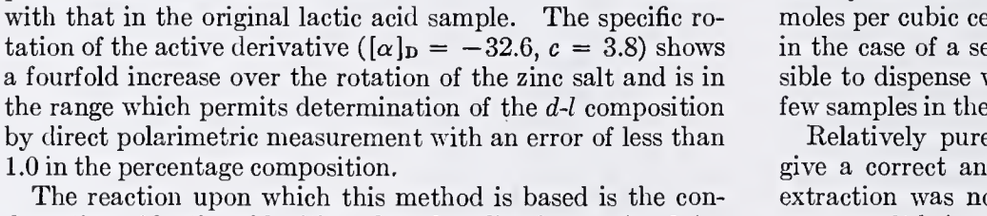
A PRACTICAL method, based on the properties of the benzimidazole derivative of lactic acid, is described for the quantitative measurement of the relative amounts of the two optical isomers in preparations of lactic acid. In the development of the present procedure, the immediate interest has been the need in research and commercial analysis for accurate optical characterization of samples of lactic acid obtained by fermentation processes.

Qualitative identification of the *d* (dextro) or "Sarco" lactic acid, *l* (levo), and *dl* (racemic) forms of lactic acid has generally been based on isolation of the zinc-*d*(or *l*)-lactate dihydrate and zinc-*dl*-lactate trihydrate, with determination of water of crystallization and optical rotation. [For clarity, and in agreement with the more general use of the term dextrolactic acid in commercial practice, the optical notations *d* and *l* are used in this paper to refer in the rotational (not configurational) sense to the forms of the acid which are dextro- and levorotating in aqueous solution.] While the zinc salt procedure is relatively satisfactory in the case of the pure isomers, or for qualitative information on mixtures, the properties of the salts are not favorable from the quantitative standpoint. Under practical conditions, if analytically pure hydrated zinc lactates are to be obtained, the crystallization of the salts from water by the addition of alcohol can rarely be carried to the point of quantitative isolation. Active-racemic fractionation is involved in the crystallization process and it is known that the *d-dl* composition of the isolated salt may differ more or less from that of the original lactic acid sample.

From the polarimetric standpoint, the specific rotation of zinc-*d*-lactate dihydrate ($[\alpha]_D = -7.9$, $c = 3$), although more satisfactory than that of the calcium salt, is still of relatively low magnitude for accurate measurement. This becomes especially apparent in consideration of *d-dl* mixtures. The previously reported appreciable variation of rotation with concentration of zinc lactate has been reinvestigated by one of the authors (R. J. D.), as reported and confirmed by Maclay, Hann, and Hudson (*7*).

In the case of the benzimidazole derivative of lactic acid, it has proved possible to isolate the benzimidazoles from *d*- and *l*-lactic acid mixtures without fractionation to obtain a pure derivative in which the ratio of *d*- to *l*-isomer is identical with that in the original lactic acid sample. The specific rotation of the active derivative ($[\alpha]_D = -32.6$, $c = 3.8$) shows a fourfold increase over the rotation of the zinc salt and is in the range which permits determination of the *d-l* composition by direct polarimetric measurement with an error of less than 1.0 in the percentage composition.

The reaction upon which this method is based is the condensation of lactic acid with *o*-phenylenediamine to give 2-(α-hydroxyethyl)-benzimidazole. Isolation of the derivative is facilitated by the insolubility of the silver salt in ammoniacal solution and regeneration of the benzimidazole by hydrochloric acid, according to the following equations:



Lactic acid — *o*-Phenylenediamine — Lactic acid benzimidazole — Ag salt

The reaction of racemic lactic acid and other α-hydroxy acids with *o*-phenylenediamine has been described by several authors (*3*, *4*, *5*, *10*). The optically active benzimidazole derivatives have not been reported. The earlier method of preparation by the heating of lactic acid with the organic base, without the addition of mineral acids, is not applicable to the *d*- and *l*-isomers because of the racemization (unpublished data) which may take place under these conditions. The satisfactory procedure for the condensation used in the following experiments, employing phosphoric-hydrochloric acid at 135° C. has been developed in conjunction with experiments on *d*-lactic acid and on carbohydrate acids (*8*, *9*). Summarized briefly, it has been proved that the stereoisomeric structure of every optically active acid studied is stable in the range from 100° to 160° C. in the presence of mineral acids. For lactic acid, with which we are concerned here, the data in Table II on known samples verify the fact of stability over a range of optical compositions.

Samples for Analysis

An aqueous sample having a lactic acid concentration of 50 per cent or more is to be preferred. For this reason, as well as because of the purification effected, an ether extraction is generally advisable as the final step in the preparation of a sample for analysis. Research preparations or commercial samples, from which major quantities of interfering substances have been eliminated by suitable methods (*1*, *2*), are acidified to thymol blue with sulfuric acid (pH approximately 2.0) and subjected to continuous extraction with ether for 24 hours. When the starting aqueous solution of lactic acid is dilute, it is convenient to add ammonium sulfate (30 grams per 100 cc.) to decrease extraction of water and to give extracts which, after removal of the ether, approximate 90 per cent lactic acid.

For the condensation it is essential that the millimoles of lactic acid present be at least equal to (preferably greater than) the millimoles of *o*-phenylenediamine. Any sample volume containing lactic acid equivalent to from one to three times the amount of *o*-phenylenediamine used will give satisfactory results. Only the approximate lactic acid titer (millimoles per cubic centimeter by alkali titration) is required and in the case of a series of similarly prepared samples it is possible to dispense with titration after experience with the first few samples in the group.

Relatively pure commercial samples, in some cases, may give a correct analysis without further purification. Ether extraction was not necessary on sample B (Table IV), the same result being obtained before and after ether purification. Also small amounts of unremoved carboxylic acids (other than lactic) do not cause large deviations in the results (Table III). In all cases the possibility of significant error due to impurities can be checked, if necessary, by analysis of the isolated benzimidazole salt for silver (40.4 per cent) or nitrogen (10.4 per cent).

Reagents

o-PHENYLENEDIAMINE (*o*-diaminobenzene), m. p. 100–102°. Photographic grades—e. g., Orthamine—are satisfactory.

Silver-Ammonia Solution. For the 5-cc. procedure, the requirement for each precipitation is 0.3 gram of silver nitrate dissolved in 0.3 cc. of water, to which are added 0.6 cc. of concentrated ammonium hydroxide in excess of the amount required to dissolve the silver oxide formed, and 0.6 cc. of 10 per cent potassium hydroxide. For a series of determinations enough silver-ammonia solution may be prepared to meet several hours' requirements (but not several days, since it may become mildly explosive) and aliquots used for each precipitation. For the 25-cc. scale five times these quantities are used.

Alcoholic Hydrochloric Acid. A solution of 8 cc. of concentrated hydrochloric acid in 92 cc. of 95 per cent ethanol.

Analytical Procedure

Two scales of operation have been found convenient. The first is based on the use of 5-cc. volumetric flasks (0.2-gram samples of lactic acid) for problems in which conservation of material is important. In the second procedure, which may be preferred in control analysis, the final volume is 25 cc.

The 5-Cc. Scale. To 0.15 gram (1.4 millimoles) of *o*-phenylenediamine in a 100 × 12 mm. test tube, add 0.2 cc. of water, 0.1 cc. of concentrated hydrochloric acid, 0.1 cc. of phosphoric acid (sp. gr. 1.7), about 0.2 cc. of ethanol, and a boiling chip. After the mixture has been brought into solution add 2 to 3 millimoles of lactic acid (about 0.25 cc. of 90 per cent acid).

Heat the reaction tube, immersed to the level of the contained solution, for 2 hours at 135° ± 5° C. (oil bath), during which time water and alcohol boil off to give a thick sirup as residue. Stir about 1 cc. of water into the warm viscous reaction mixture and transfer into a 25-cc. flask with about 5 cc. of water. Add 3 cc. of ethanol, 9 cc. of 10 per cent potassium hydroxide, and a little (about 0.05 gram) decolorizing carbon (Nuchar C-250 or Darco). Filter with suction through an asbestos pad (Woolly C) and wash the pad twice with 0.5-cc. portions of ethanol.

Transfer the filtrate to a 50-cc. flask and add 8 cc. of concentrated ammonium hydroxide. Allow the solution to stand for 20 minutes before precipitating the lactic acid benzimidazole as the anhydrous crystalline silver salt by the dropwise addition of silver-ammonia solution equivalent to 0.3 gram of silver nitrate.

Shake the flask for about 1 minute to decompose any aggregates present and allow the salt to settle for 10 minutes. Discard the supernatant solution by decantation and filter the salt on a small Büchner funnel with gentle suction. Wash three times with dilute ammonium hydroxide (10 cc. of concentrated ammonium hydroxide in 200 cc. of water), three times with ethanol, and once with ether. During the washing the suction should be applied and released in such a manner that the salt is not pulled dry (filter cake should not crack) until the final ether wash.

Dry the salt in a vacuum desiccator for about 30 minutes (with well-washed samples, air-drying for the same period of time gives satisfactory results). Transfer the dried salt to a weighed 5-cc. volumetric flask and reweigh. Add 2 cc. of ethanol, shake gently (in upright position) to suspend the silver salt uniformly, and add to the suspension 2 cc. of alcoholic hydrochloric acid solution. Shake well for about a minute. Add 0.15 cc. of concentrated ammonium hydroxide, make up to volume with ethanol, stopper the flask, and shake. After the halide has settled for a few minutes, shake the supernatant solution into the neck of the flask once or twice to wash down any loose particles of silver chloride. Centrifuge the flask for 5 minutes at about 2000 r. p. m. (standard cups for 50-cc. tubes, with cotton packing, are satisfactory). Without disturbing the residue, pipet a sample of the clear supernatant solution into a semimicro 2-dm. polariscope tube (capacity of 2 to 3 cc.). From the rotation (angular degrees) of this solution and the weight of silver salt, w, the per cent of *d*-lactic acid in the original sample is obtained as follows:

$$\text{Per cent } d = 50 - \frac{6.37\alpha}{w}$$

The rotation of the benzimidazole derivative is in the opposite direction to that of the free acid, as is the case with other derivatives of the isomeric lactic acids.

Reprecipitation. If measurement of the rotation is rendered difficult by the presence of color in the alcoholic solution, two possible causes may be considered. The presence of unreacted *o*-phenylenediamine, due to failure to add sufficient lactic acid for the condensation, will give rise to interfering color as a result of oxidation by the silver-ammonia solution. Or failure to remove color-producing impurities from the original lactic acid sample may be involved. A repetition of the condensation, using a correct amount of lactic acid, will eliminate the first effect. Purification of the acid sample by ether extraction, in general, prevents possible interference due to the second cause.

If for any reason a second condensation is not convenient, a rotation with the colored benzimidazole preparation can usually be realized by carrying out a reprecipitation of the silver salt Decolorize the alcohol solution of the benzimidazole (about 5 cc.) with a little carbon, using 1 cc. of ethanol in washing, and add 2 cc. of 10 per cent potassium hydroxide, 8 cc. of water, and 5 cc. of concentrated ammonium hydroxide. Precipitate with silver-ammonia solution (0.3 gram of silver nitrate) and proceed as in the first determination.

The 25-Cc. Scale. Use 10 to 15 millimoles of lactic acid (about 1 cc. of 90 per cent acid), with five times the quantities of reagents and solvents prescribed above, 15-cm. (6-inch) test tubes, and correspondingly increased flask sizes. Standard 2-dm. saccharimeter tubes (10- to 15-cc. capacity) can be used. The factor in the equation for per cent *d*-lactic acid becomes 31.85 instead of 6.37. Centrifuge cups designed for Babcock bottles are suitable for the usual 25-cc. volumetric flasks. If centrifuging is not convenient, the silver chloride separation may be accomplished on this larger scale, after the solution has been made to volume, by rapid filtration through a dry paper directly into the saccharimeter tube. Table IV presents results obtained on the same sample by both procedures. For most practical purposes on this scale satisfactory readings may be obtained with a saccharimeter and converted to angular degrees.

Example of Data and Calculation

Lactic acid benzimidazole, m. w. 162 — *d*-benzimidazole $[\alpha]_D = -32.6°$
Ag-salt of benzimidazole, m. w. 269 — Volume = 25 cc.
Conversion factor = 0.602 — Tube length = 2 dm.

Weight of flask + Ag-salt = 16.6564
Weight of flask = 15.1075
Weight of silver salt, w = 1.5489

Polarimeter: $\alpha = -1.45$ A.
Saccharimeter:
$\alpha = -4.2°$ Ventzke
$= -4.2 \times 0.3466$
$= -1.46$ Å.

$$[\alpha]_D = \frac{100\alpha}{lc} = \frac{100\alpha}{2 \times \frac{w}{25} \times 0.602 \times 100} = \frac{20.76\alpha}{w}$$

$$\text{Per cent } d = 100\left[\frac{[\alpha]_D}{-32.6} + \frac{1}{2}\left(1 - \frac{[\alpha]_D}{-32.6}\right)\right] = 50 - \frac{31.85\alpha}{w}$$

$$\text{Per cent } d = 50 - \frac{31.85\ (-1.45)}{1.549} = 79.8 \text{ (per cent } l = 20.2)$$

The per cent *d* calculated in these equations represents the total *d*-isomer content of the lactic acid under analysis. This calculation is to be distinguished from the expression of the composition of a sample in terms of *d* (or *l*) and *dl* (racemic). The total *d*-isomer content, as expressed in the above example, is equal to the excess *d*-isomer present plus one half of the inactive *dl*. Observance of the correct sign for α in the above expression consistently gives the result in terms of per cent *d* (per cent $l = 100 - d$).

Discussion

The benzimidazole derivative of lactic acid shows the general amphoteric properties of this type of heterocyclic compound (*6*, *9*). With acids it forms salts, most of which are water-soluble—e. g., hydrochloride, acetate, etc. It also forms soluble salts with strong alkalies, but insoluble salts with silver, zinc, and copper. The free base crystallizes readily (but not quantitatively) from water and may be isolated by the addition of a weak base (ammonium hydroxide) to the acidic aqueous solution obtained in the condensation. The lacto-benzimidazole is soluble in many organic solvents, and the ethanol added in this procedure prevents crystallization of the free base from the ammoniacal solution.

Solubility of Silver Salt. The benzimidazole is isolated as the crystalline silver salt rather than the free benzimidazole in this procedure because of the advantage of essentially quantitative yield and consequent absence of *d-dl* fractionation. Selective precipitation of the silver-benzimidazole in the presence of chloride ion is made possible by the insolubility of the salt of the heterocyclic base in the excess of ammonium hydroxide which prevents silver chloride pre-

cipitation. It has been observed that the ammonium salts of acids—e. g., ammonium chloride—have a solubilizing effect. In order to prevent fractionation of the isomers by incomplete precipitation, the solvent effect of ammonium salts has been blocked by the addition of an excess of potassium hydroxide for neutralization of mineral acids and excess lactic acid.

TABLE I. VARIATION OF $[\alpha]_D^{24}$ WITH CONCENTRATION

(K_c represents specific rotation of pure *d*-benzimidazole from lactic acid at a given concentration, under conditions used in preparation of solution from a sample of silver salt.)

Silver Salt, 5-Cc. Procedure	Silver Salt, 25-Cc. Procedure	Benzimidazole Concentration	K_c[a]
Gram	*Grams*	%	
0.25–0.26	1.25–1.30	3.1	−32.3
0.27–0.28	1.35–1.40	3.3	−32.4
0.29–0.30	1.45–1.50	3.6	−32.5
0.31–0.32	1.55–1.60	3.8	−32.6
0.33–0.34	1.65–1.70	4.0	−32.7
0.35–0.36	1.75–1.80	4.3	−32.8

[a] Values of K_c have been found essentially constant over temperature range 15° to 25° C. Rotations can therefore be taken over normal ranges of room temperature and thermostatic control is not necessary.

Under the conditions given in the experimental section, without attempt for absolute manipulative recovery, the isolated yields of silver salt from a standard solution of crystalline benzimidazole were, in triplicate, 97.6, 97.4, and 98.0 per cent of theory (analysis for silver: calculated, 40.1; found, 40.3). The optical composition of this sample was 70.6 per cent *d* and the found values for the three precipitates were 71.0, 70.8, and 70.0.

The principles for the solubility of the silver salt find application again in the final solution of the sample for polarization. In this case the solubility of the silver salt in the presence of ammonium chloride permits neutralization of hydrochloric acid by an excess of ammonium hydroxide in the presence of silver chloride without precipitation of silver-benzimidazole. The trace of silver ammonia which may be formed is without effect.

SPECIFIC ROTATION. In the case of the benzimidazole derivative of *d*-lactic acid, the variation of specific rotation with concentration is measurable but relatively small (Table I). In the equations for calculation of per cent *d*, an average value of 32.6 has been used and the error thus introduced is within the experimental error of the method, provided the sample weights are kept within the listed range (benzimidazole concentration 3 to 4.5 per cent).

When the weighed sample of silver salt contains a mixture of the *d*- or *l*-isomer with the *dl* form, the concentration of the active component may be less than 1 per cent. However, in the calculation of the composition, the rotation of the pure isomer at the 4 per cent concentration (−32.6) is still applicable. In such mixtures the specific rotation of the excess *d*- or *l*-isomer is essentially a function of the total benzimidazole concentration. If the concentration of *d*-lacto-benzimidazole (from the silver salt) is varied while the total benzimidazole concentration is held constant at 4 per cent by the addition of the inactive *dl*-derivative, the specific rotation of the *d*-benzimidazole used remains nearly constant. For example:

$[\alpha]_D = -32.7$, *d*, $c = 4$ and *dl*, $c = 0$; $[\alpha]_D = -32.3$, *d*, $c = 1$ and *dl*, $c = 3$; but $[\alpha]_D = -31.3$ when *d*, $c = 1$ and *dl*, $c = 0$

If desired, the concentration factor may be incorporated in the calculations to compensate for variations in sample weight. The value for K_c corresponding to the weight of silver salt used may be taken from Table I (or an extrapolation thereof).

$$\text{Per cent } d\text{-lactic acid} = \frac{100}{2}\left[1 + \frac{[\alpha]_D}{K_c}\right]$$

The values in Table I represent the rotations of the active derivative in the particular solvent (approximately 90 per cent ethanol) obtained under the experimental conditions of this method. In pure 95 per cent ethanol the rotation is of similar magnitude ($[\alpha]_D = -32.8$, $c = 3$). In aqueous acids the rotation is lower ($[\alpha]_D = -14.7$, $c = 2$, in 5 per cent citric acid).

The technique of precipitating the silver chloride in the volumetric flask and making to volume before filtering (or centrifuging) is preferable to the alternative order of the operations. Experience with silver chloride precipitation followed by filtration, washing of the precipitate, and adjustment of the combined filtrates to the desired volume, has invariably shown significantly low recoveries. It does not appear possible, within the practical limits of solvent volume for polarimetric measurement, to obtain a quantitative washing of the silver chloride precipitate. The centrifuge technique has regularly given recoveries of 99 per cent or higher.

The actual minor volume error (approximately 0.7 per cent) introduced by the presence of the solid phase in the volumetric flasks is present in both the analysis and in the preparation of Table I. This factor therefore cancels out in the equation for per cent *d*-lactic acid and does not require consideration.

TABLE II. ANALYSES OF KNOWN MIXTURES OF LACTIC ACID FOR OPTICAL FORM

Lactic Acid	Benzimidazole Ag-Salt	α, Angular Degrees	*d*-Lactic Found	Deviation
	Gram		%	
Pure *d*-lactic (100% *d*)	0.3151	−2.46	99.7	−0.3
	0.3335	−2.63	100.2	+0.2
	0.3117	−2.44	99.9	−0.1
	0.3326	−2.62	100.2	+0.2
	0.2886	−2.26	99.9	−0.1
Known mixture No. 1 (74.7% *d*)	0.3263	−1.30	75.4	+0.7
	0.3430	−1.38	75.6	+0.9
	0.3159	−1.26	75.4	+0.7
	0.3436	−1.36	75.2	+0.5
Known mixture No. 2 (40.0% *d*)	0.3324	+0.51	40.2	+0.2
	0.3221	+0.49	40.3	+0.3
	0.3284	+0.50	40.3	+0.3
	0.3054	+0.48	40.0	0.0
	0.3177	+0.47	40.6	+0.6
	0.3322	+0.50	40.4	+0.4
Known mixture No. 3 (14.0% *d*)	0.3112	+1.74	14.4	+0.4
	0.3181	+1.76	14.8	+0.8
	0.3171	+1.76	14.6	+0.6
	0.3103	+1.74	14.3	+0.3
	0.3147	+1.75	14.6	+0.6

ACCURACY. The analyses in Table II show application of the method to lactic acid samples of known optical composition. Known mixtures were prepared gravimetrically from zinc-*d*-lactate dihydrate ($[\alpha]_D = -7.6$; *c*, 4; $H_2O = 12.83$ per cent), zinc-*l*-lactate dihydrate ($[\alpha]_D = +7.5$; *c*, 4; $H_2O = 12.84$ per cent), and zinc-*dl*-lactate trihydrate ($[\alpha]_D = 0.0$, *c*, 1.5; $H_2O = 18.29$ per cent). The salt mixtures were dissolved in water, acidified, and extracted with ether as in the experimental procedure. The values found for per cent *d*-isomer indicate that, in general, the observed per cent will not vary by more than 1.0 from the actual composition. In Table II the observed percentage composition shows a maximum deviation of 0.9 and mean deviation of 0.4 for the representative series of samples listed.

TABLE III. EFFECT OF PRESENCE OF ACETIC AND SUCCINIC ACIDS

Lactic Acid	Benzimidazole Ag-Salt	α, Angular Degrees	*d*-Lactic Found
	Gram		%
Commercial edible	0.3153	−2.44	99.3
	0.3029	−2.34	99.2
10% HOAc added	0.3395	−2.52	97.3
	0.3375	−2.49	97.0
50% HOAc added, steam-distilled before analysis	0.3423	−2.64	99.1
	0.3235	−2.50	99.2
	0.3096	−2.39	99.2
10% succinic acid added	0.3049	−2.24	96.8
	0.2767	−2.07	97.7

Effect of Presence of Other Carboxylic Acids. Under the conditions used in this method the condensation with *o*-phenylenediamine is general for the carboxyl group in the aliphatic series. The samples should be free, therefore, from major quantities of acids other than lactic. Small amounts of acetic acid or succinic acid, however, can be present without significantly altering the results. It may be noted that 10 per cent of either acid added to a commercial sample lowered the analytical figures by only 1 to 2 per cent (Table III). The small magnitude of this interference is due to the difference in the rates at which α-hydroxy acids and unsubstituted aliphatic acids react with *o*-phenylenediamine. The reaction rate in the case of lactic acid is relatively high.

Table IV. Analyses of Commercial and Experimental Samples

Lactic Acid	Benzimidazole Ag-Salt	α, Angular Degrees	*d*-Lactic Found
	Grams		%
Commercial edible, ether extracted	0.3380	−2.53	97.7
	0.3494	−2.60	97.4
Without ether extraction	0.3333	−2.46	97.0
	0.3260	−2.41	97.1
25-cc. procedure	1.7160	−2.57	97.7
	1.6601	−2.48	97.6
Experimental fermentation	0.3230	−1.53	80.2
	0.3213	−1.54	80.5
25-cc. procedure (centrifuged)	1.5489	−1.46	80.0
	1.3507	−1.25	79.5
25-cc. procedure (filtered)	1.6597	−1.55	79.7
	1.4786	−1.39	79.9
Experimental fermentation	0.3191	−1.08	71.6
	0.3211	−1.09	71.6
25-cc. procedure	1.5623	−1.08	72.0
	1.5698	−1.08	71.9
Experimental fermentation	0.2978	−0.32	56.8
	0.3080	−0.32	56.6
	0.3024	−0.32	56.7
Experimental fermentation	0.3479	+2.18	10.1
	0.3446	+2.16	10.1

Commercial and Experimental Samples. Table IV lists representative analyses on a variety of lactic acid preparations. In several cases analyses have been made by different operators employing different scales of procedure and the results are found to be in agreement.

Summary

A quantitative procedure for the measurement of the relative amounts of the two stereoisomers in preparations of lactic acid is described. It is applicable, with an error of less than 1.0 in the percentage composition, to samples containing as little as 0.2 gram of lactic acid. To meet the requirements of variations in the available sample size and equipment, two scales of operation are given. The procedure depends on the condensation of lactic acid with *o*-phenylenediamine at 135° C. in the presence of phosphoric and hydrochloric acids to form 2-(α-hydroxyethyl)-benzimidazole. The derivative is isolated by quantitative precipitation as the crystalline silver salt. Regeneration of the benzimidazole gives a solution whose specific rotation is used for the calculation of the *d-l* composition of the original lactic acid sample. The use of the benzimidazole derivative of lactic acid offers the following advantages over the zinc salt: a fourfold increase in rotation ($[\alpha]_D = -32.7$), negligible variation of rotation with concentration, and absence of fractionation of isomers during the preparation and isolation of the derivative.

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Fastness to Atmospheric Gases of Dyes on Cellulose Acetate Rayon

AT A MEETING of the Research Committee of the American Association of Textile Chemists and Colorists the following method was adopted as a tentative method.

Test Specimens. Four specimens shall be cut from the fabric to be tested. One specimen shall be washed in a soap solution (containing 5 grams of 88 per cent neutral soda soap per liter of water of approximately zero hardness) for 15 minutes at 37.78° C. (100° F.), rinsed in warm water, and allowed to dry in the air. One specimen shall be immersed in cold Stoddard solvent for 15 minutes, squeezed, and allowed to dry in the air. One specimen shall be immersed in cold perchloroethylene for 15 minutes, squeezed, and allowed to dry in air. The fourth specimen shall be retained in its original condition.

Apparatus. The apparatus shall consist of an enclosed metal chamber, connected by a metal pipe to a lighted gas burner in such a manner that the burnt gases from the burner pass directly through the chamber. Either natural or manufactured gas may be employed. It is strongly recommended that a motor-driven fan be installed in the chamber to keep the fumes well distributed, and that a grid of iron wire be placed over the gas burner, as this will accelerate the production of oxides of nitrogen, which are responsible for the fading of the dyes.

Procedure. Samples of the four specimens, each measuring at least 2.5 cm. (1 inch) square, shall be suspended freely in the chamber together with a "control sample" of a standard acetate rayon dyeing of known sensitiveness to gas fumes. [The control sample shall consist of a 1 per cent dyeing of Celanthrene Brilliant Blue FFS (or its equivalent) on acetate rayon satin.] The gas burner shall be lighted, and the flame adjusted so that the temperature in the chamber does not exceed 60° C. (140° F.). The sample shall remain in the chamber until the control sample shows a change of shade corresponding in degree with that of a "standard fading". [The standard of fading shall consist of a silk fabric dyed with stable dyes to imitate the color of the control sample after it has been exposed to average atmospheric conditions for a period of 6 months. Samples of both the control sample and standard of fading are available from C. A. Seibert, P. O. Box 386, Wilmington, Del.] The samples shall then be removed from the chamber and immediately compared with the respective unexposed portions.

Evaluation. A sample which shows no alteration of shade shall be considered to have satisfactory resistance to atmospheric gases. A sample which shows as much alteration of shade as the control sample must be regarded as definitely sensitive to atmospheric gases.

Rapid and Accurate Determination of Cadmium

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THE separation of zinc and cadmium has long been a stumbling block in rapid and accurate analyses in the nonferrous metallurgical fields, and laboratories having a large number of cadmium determinations have sought a more rapid and accurate method. The standard procedures of the present day call for the separation of zinc and cadmium by repeated precipitations with hydrogen sulfide or by electrolysis under carefully controlled conditions of pH and current density, involving the use of various addition agents. Because of the consanguinity of zinc and cadmium, these methods are not satisfactory.

Studies have shown that the precipitation of cadmium cannot give a complete separation at any pH (*5*, *7*). Repeated precipitations can only give a closer approximation, with each handling giving greater chance for error. Other objections to this method are the length of time and amount of manipulation required, the toxic nature of the hydrogen sulfide gas, and its destructive effect on the laboratory equipment.

The separation of cadmium and zinc by electrolysis has been extensively studied. The mass of variant literature gives a picture of the confusion in this field. Present methods have been shown to give only partial separation (*1*). As yet no electrolytic method has been found completely satisfactory to the large routine laboratory.

Recent developments in the organic-metallurgical field have uncovered a number of substances that quantitatively precipitate cadmium with no effect on zinc (*2*, *4*). Most of these are very expensive. However, in 1937 Korenman (*3*) suggested the precipitation of cadmium from a cadmium-zinc solution by a brucine–potassium bromide mixture, its subsequent filtration, washing, drying, and weighing. The objectionable features of the method were the long period of precipitation, inability to wash clean, and the tendency of the precipitate, due to its great bulk, to decompose, volatilizing the bromine, before completely drying. Its advantages were the completeness of separation and the cheapness and cleanliness of the chemical.

Experiments have shown that if the cadmium is precipitated from the cadmium-zinc solution with a brucine sulfate–potassium iodide mixture and then washed by special means, the precipitate can be dissolved and the cadmium determined by titrating the iodine present, giving accurate and rapid results if carefully carried out.

Experimental Work

A solution containing cadmium-zinc salts, and freed from lead, copper, and iron, is reduced to a volume of 50 cc., if previous manipulations have resulted in excess volume. The original sample is so taken as to give 20 to 50 mg. of cadmium in the solution. It may be necessary to take aliquot portions of the original solution to get this correct cadmium content. For every milligram of cadmium believed present, 1.5 cc. of a 1 per cent brucine sulfate solution are added, followed immediately by 1.5 cc. of a 10 per cent potassium iodide solution. The solution is well stirred and allowed to stand for 10 minutes, then filtered rapidly through a Büchner funnel using suction. The precipitate is first washed with a 50–50 mixture of the brucine and iodide solutions, then with an organic solvent such as a 1 to 4 mixture of ethanol and toluene until free from potassium iodide. It is then dissolved by heating in water and the iodine content of the organic iodide is determined in any convenient manner. The cadmium content of the sample may be determined by comparison with the iodine value of standards previously determined.

Elements, usually present in nonferrous metallurgy, that interfere with the determination are iron, copper, lead, and antimony. These elements are normally removed during any analysis and will cause no additional work when this method is used. Precipitation should be carried out in a neutral or slightly acidic solution. However, no free nitric acid or other strong oxidizing agent should be present, as it would liberate free iodine from the iodide as well as destroy the brucine. Free ammonium hydroxide should not be present, though ammonium salts do not interfere.

Unlike many other methods, the brucine-iodide precipitation method covers the entire range of zinc-cadmium ratios. Table I shows the results obtained by analysis of mixtures made up to represent various samples in the processes of the zinc and cadmium smelters.

TABLE I. SEPARATION OF ZINC AND CADMIUM

Zn Present *Grams*	Pb Present *Gram*	Cd Present *Gram*	Cd Found *Gram*	Error *Mg.*	Ratio of Zn to Cd
40.005	0.500	0.0200	0.0201	+0.1	1000–1
0.0200	...	0.0201	0.0201	0.0	1–1
0.100	...	0.0250	0.0249	−0.1	4–1
0.270	...	0.0302	0.0302	0.0	9–1
0.003	0.300	0.0301	0.0302	+0.1	1–10
2.000	...	0.0400	0.0402	+0.2	50–1
0.100	0.075	0.0502	0.0503	+0.1	2–1
0.0025	0.022	0.0500	0.0500	0.0	1–20

Standards consisted of commercial cadmium metal running 99.8 per cent cadmium and 0.1 per cent zinc. They were determined in about half an hour. Zinc spelter of the ordinary grades may be run in less than 2 hours, compared to a previous time required of approximately 7 hours. Below is given the detailed procedure for the analysis of a raw ore running 60.0 per cent zinc and 0.4 per cent cadmium with many impurities. The time required is one third that of a hydrogen sulfide determination of the same accuracy with the same sample.

The procedure includes steps made necessary by the complex nature of the raw ores. Procedures for ordinary cadmium samples are normally simple and rapid.

Procedure for a Sulfide Ore

SOLUTION OF SAMPLE. Weigh a 5.00-gram sample into a 400-cc. beaker, add 15 cc. of concentrated hydrochloric acid, and boil until all hydrogen sulfide has passed off. Add 15 cc. of concentrated nitric acid and bake. Cool, add 30 cc. of concentrated hydrochloric acid and 5 grams of ammonium chloride, and boil until the volume is reduced to 5 to 10 cc.

REMOVAL OF IRON. Dilute to 200 cc. with water and add ammonium hydroxide until the iron is all precipitated, adding sufficient to redissolve the cadmium and zinc. Boil to granulate the precipitate, then filter, washing the beaker three times, using a drop of ammonia with the wash water each time. Wash the precipitate four times with hot water. (If the iron content is above 5 per cent, dissolve, reprecipitate, filter, and wash as above.) Neutralize the filtrate with concentrated hydrochloric acid to the methyl orange end point, adding 7 cc. in excess.

REMOVAL OF COPPER. Add 3 to 5 grams of granulated C. P. lead and boil for 10 minutes to precipitate out the copper. Add another full portion of lead and again boil, then test for completeness by adding a few fresh grains of lead. Remove from the heat and decant the liquor. Wash the lead and copper three times by decantation with hot water, adding the washings to the liquor.

SEPARATION OF THE CADMIUM. Add 5 grams of pure magnesium dust to the solution and boil for 3 minutes. Filter through a No. 4 Whatman paper, washing paper and precipitate until free from acid. Repeat the precipitation by adding another portion of magnesium to the filtrate, again boiling, filtering, and washing. Transfer the precipitate to a 150-cc. beaker using 1 to 1 nitric acid and a fine spray of water. Dissolve the sponge in 5 cc. of concentrated nitric acid and take to dryness. Add 15 cc. of concentrated sulfuric acid and again take to dryness. Dissolve with 25 cc. of water and filter off the lead sulfate, using a fine paper. Wash the beaker twice and the paper four times, using only a small volume of water.

Add 30 cc. of the brucine sulfate solution followed immediately by 30 cc. of the potassium iodide solution. Stir well and let settle for 10 minutes. Filter through a 5-cm. (2-inch) Büchner funnel, using moderate suction and a very fine paper. Wash the beaker with a 50–50 mixture of the stock solutions (mixed immediately before use) three times and the paper three times with a 1 to 4 mixture of ethanol and toluene.

Estimation. Wash the precipitate and paper back into the beaker with water, making up to 100 cc. Heat until the precipitate has dissolved. Add 5 cc. of a 0.5 per cent solution of eosin Y as an indicator and titrate to the absorption end point with 0.03 *N* silver nitrate (*6*). The titration value is compared to that of a standard test to give the amount of cadmium in the sample.

Notes

In making up the brucine sulfate solution, the addition of sulfuric acid up to 5 per cent is helpful in the solution of the brucine and in the adjustment of the acidity during the precipitation. Care must be taken, however, not to oxidize the brucine. For best results, a fresh solution of brucine sulfate should be made every day.

Excessive volume for the precipitation may cause a colloidal precipitate. Difficult filtrations may be aided by judicious choice of filter papers and filter aids.

If there is a large mass of precipitate, it may hold some of the iodide solution, raising the titer and affecting the factor. It may be necessary to dissolve and reprecipitate the cadmium. The work requires only a few minutes and may be well worth the time

As in most analytical procedures, directions must be minutely followed, and a definite technique established. Careless work or minor differences in procedure may vitiate all results. Short cuts or the removal of additional elements will doubtless cause a different titer value. Careful work and an established technique, always qualities of a good analyst, are fully repaid by extremely accurate and rapid determinations of cadmium that will effect savings in time and money, especially in the large routine laboratories of smelters and metal-working plants.

Acknowledgment

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Detection of Sodium Alginate in Dairy Products

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AMONG the thickening agents being used at the present time in the dairy industry is the sodium salt of alginic acid, derived from certain seaweeds and put on the market in various forms. The product which is probably most widely used in this country is sold under the trade name of Dariloid, in which sodium alginate is mixed with other substances including dextrin, sucrose, and a phosphate. Other commercial products may consist of the pure alginate.

The present investigation was undertaken because of a lack of a specific qualitative test for alginic acid. The test is needed because of the widespread use of alginates in several dairy products, especially ice cream; and because of the possibility of illegal use in milk, cream, or other dairy products.

Inasmuch as the commercial product is likely to contain dextrin, a quick preliminary test for dextrin may be used to establish the probable presence of alginic acid in milk or cream. Likewise, milk or cream containing the commercial product is likely to give a positive Rothenfusser test (*1*) for sucrose.

Tests

Optional Preliminary Test for Dextrin. Take 10 cc. of milk or cream, dilute with 10 cc. of distilled water, and add 10 cc. of a 20 per cent solution of trichloroacetic acid. Shake vigorously for 1 minute, let stand for 5 minutes, and filter. Make the filtrate alkaline with ammonium hydroxide, evaporate on a steam bath to a volume of about 1 cc., filter into a test tube, cool to room temperature, and add 0.05 *N* iodine solution cautiously to the filtrate, a small drop at a time. A violet to reddish-violet color indicates the possible presence of 0.1 per cent or more of Dariloid.

Specific Test for Alginic Acid. To 20 grams of milk, cheese, cream, or ice cream add a volume of concentrated hydrochloric acid approximately equal to the water content of the sample taken. Shake thoroughly, add a little sand (acid-washed and ignited), bring to a boil, and boil 30 seconds with frequent shaking. Transfer to a 50-cc. centrifuge tube with the aid of 10 to 20 cc. of alcohol. Centrifuge 10 minutes, or until the solid matter forms a compact cake at the bottom of the tube, and decant as much of the supernatant liquid as possible from the solid matter, taking care not to lose any of the solids. The centrifuge should be warm enough to keep the fat liquid and should be allowed to slow down without braking, to avoid stirring up the solids. In some cases, the fat mixed with part of the solids forms at the surface a dense cake which must be punctured with a stirring rod to allow removal of the liquid. The decantation is best carried out against a white background to facilitate observation of the solids through the dark solution.

Wash the solids repeatedly with 75 per cent ethyl alcohol by shaking, centrifuging, and decanting as above, until the wash solution is neutral to litmus paper and is colorless. Then wash twice with ether in the same manner.

Evaporate the last traces of ether by warming the tube in a beaker of hot water and directing a current of air into it. Dissolve the residue so far as possible in 10 cc. of 0.1 *N* sodium hydroxide by shaking a few seconds. Filter, wash the tube and paper with a few cubic centimeters of water, and to the filtrate add an equal volume of 95 per cent ethyl alcohol. Centrifuge 10 minutes, decant the supernatant liquid, and wash the solids with 75 per cent alcohol by centrifuging and decantation until the wash solution is neutral to litmus paper. The solution should be centrifuged and decanted even when it appears clear, as small amounts of gum are invisible beforehand. If the separated gum is now white and free from any yellow or brown color, pass over the next paragraph.

Suspend the gum in 10 cc. of distilled water and add 0.1 *N* sodium hydroxide dropwise, with shaking, until the solution is just alkaline to litmus paper. Add 6 cc. saturated magnesium nitrate solution and shake thoroughly. Centrifuge 10 minutes and decant the supernatant liquid from any precipitate into another centrifuge tube. Discard the precipitate and make the solution acid with a drop of concentrated hydrochloric acid.

Centrifuge, decant, and wash the precipitate with 75 per cent alcohol as before, until neutral.

Dry the gum by warming the tube with hot water and blowing air into it until no odor of alcohol is perceptible. Dissolve as far as possible by shaking with 0.15 cc. of 0.1 *N* sodium hydroxide, add 1 cc. of sulfuric acid reagent, shake thoroughly, and let stand at room temperature.

[The sulfuric acid reagent was prepared by precipitating ferric hydroxide from ferric chloride solution with ammonium hydroxide, washing the ferric hydroxide until neutral, drying on the steam bath, and finally saturating concentrated sulfuric acid with the dry ferric oxide by allowing the two to stand in contact for several days. The clear acid solution was then decanted from any excess ferric sulfate.]

Within a few minutes to several hours, depending on the amount of alginic acid present, the solution will develop a pink color deepening through cherry red to magenta, and finally becoming a deep purple. When the amount of gum is 0.5 mg. or less, the purple color is permanent for at least 1 week, but with larger amounts the color eventually changes to a brown-black.

Table I shows the time required for development of the color with various amounts of the gum.

Discussion

By means of this test, it is easily possible to detect 0.2 per cent of sodium alginate or Dariloid in dairy products.

The following substances do not interfere with the test: starch, gelatin, Irish moss, agar, gum tragacanth, India gum, locust bean gum, gum arabic, and formaldehyde. The test has been found satisfactory with a 0.2 per cent solution of Dariloid in milk, heavy cream, cream cheese, and vanilla, strawberry, chocolate, and maple walnut ice cream.

Table I. Development of Color

Weight of Dry Gum *Mg.*	Time for Development of Violet Tinge	Time for Development of Deep Purple Color
0.04	No color developed	No color developed
0.1	Overnight	Not reached after standing one week
0.3	4 hours	Overnight
0.5	4 hours	Overnight
1.0	10 minutes	3 hours
3.3	2 minutes	1 hour

In order to make sure that the substance which gives the color test with the sulfuric acid reagent was derived from the sodium alginate and not from any accompanying impurity, a sample of sodium alginate, which was represented as being substantially pure, was obtained from the Kelco Company. This product was dissolved in water and precipitated by the addition of an equal volume of concentrated hydrochloric acid. The mixture was then boiled for 1 minute and the residue washed free of acid by repeated centrifugalization and decantation. The residue was then dissolved in just sufficient 0.1 *N* sodium hydroxide and the whole process repeated five more times. The residue from the final acid precipitation was assumed to be free of any contaminants and still gave the color test.

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Determination of Menthol in Oil of Peppermint

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EACH year it is necessary for the author's laboratory to analyze several hundred samples of oil of peppermint within the short period which comprises the harvesting season. It has, therefore, been the constant aim to devise a method which would permit more rapid analyses without sacrificing accuracy. The method of Power and Kleber (*6*), which is generally used in the aromatics industry and is the official procedure of the United States Pharmacopoeia, is known to be erroneous and to give variable results in different laboratories; however, none of the modifications proposed thus far has been entirely satisfactory. This procedure is known to indicate as alcohols such nonalcohol constituents as aldehydes, amines, phenols, esters, and some of the more unstable terpenes which are acetylated along with the alcohols when that method is used. The present are included during saponification. During the course of research this method has been thoroughly investigated and, as a basis for further conclusions, it is believed advisable to review briefly the possible sources of error in the order in which they occur during the analysis of an oil. These discrepancies include those observed in the author's laboratory as well as those reported in the literature.

In a report upon the analysis of oil of peppermint, Nelson (*5*) states that "the proper care of the sample previous to analysis is highly important: all reagents used must be of the best quality and the sodium acetate used as a catalyst must be absolutely anhydrous."

It has been observed here that acetic anhydride must be used within a short time after the container is opened; otherwise, deterioration of the anhydride, due to its hygroscopic nature, will be evidenced by low results in alcohol analyses. Frequent standardization of solutions, especially of the alcoholic potassium hydroxide, is necessary to ensure consistent results. It has been found necessary to use hot solutions when washing the acetylated oil to hydrolyze the excess acetic anhydride completely. No evidence of hydrolysis of the acetylated oil has been found if this procedure is used and the dried acetylated oil has always been neutral.

The author has long stressed an exact 1-hour saponification time, having observed that variations in this factor caused marked differences in results. Recently Baldinger (*1*) conducted a time study, varying the length of both acetylation time and saponification time with each sample. The interpretation of the results of this study is summarized as follows: "It would seem that the time of acetylation may vary within broad limits, while the time of saponification had best be varied between 45 and 60 minutes. While experimental evidence is not yet available to prove the contention, it is believed that resinification or polymerization of certain constituents is induced by prolonged heating with potassium hydroxide and that some base is used up, thereby leading to erroneous results."

Redemann and Lucas (*7*) have observed that more rapid hydrolysis of esters results if diethylene glycol is substituted for ethyl alcohol as a solvent for potassium hydroxide. This has been verified by Hall, Holcomb, and Griffin (*4*), who applied this method to the analysis of the isomers of menthol.

In the titration of the saponified oil, variations occur due to differences in the estimation of the end point by different analysts. Badly oxidized or poor quality samples are often difficult to analyze consistently, owing to discoloration during saponification. This interferes with the observation of the end point when phenolphthalein is used as an indicator.

The method of Delaby, Sabetay, and Breugnot (*2*) for the determination of free alcohols in the sandalwood oils gives results from 5 to 8.5 per cent lower than those obtained by Power and Kleber. These authors traced this divergence to the fact that

Table I. Free Menthol Determinations, Showing Effects of Variations in Acetylation and Saponification Periods

Sample	Free Menthol Present	Acetylation Period	Free Menthol Found in Saponification Period of: 0.5 hour	1 hour	1.5 hours	2 hours	Av.
	%	*Hours*	%	%	%	%	%
		0.5	0.2	0.1	0.1	0.5	0.2
		1	0.1	0.1	0.1	0.2	0.1
1	0.0	1.5	0.1	0.1	0.3	0.3	0.2
		2	0.2	0.1	0.1	0.3	0.2
		Av.	0.1	0.1	0.2	0.3	0.2
		0.5	13.2	13.1	13.3	13.9	13.4
		1	13.0	13.0	13.3	13.3	13.1
2	12.6	1.5	13.1	13.3	13.3	13.5	13.3
		2	13.0	13.3	13.3	13.7	13.3
		Av.	13.1	13.2	13.3	13.6	13.3
		0.5	26.2	25.9	26.2	27.3	26.4
		1	25.9	26.0	26.3	26.6	26.2
3	25.5	1.5	26.2	26.6	26.0	26.7	26.4
		2	26.1	26.3	26.4	26.7	26.4
		Av.	26.1	26.2	26.2	26.8	26.3
		0.5	37.9	38.1	38.8	28.7	38.4
		1	38.2	38.4	38.6	38.4	38.4
4	37.5	1.5	37.9	38.2	38.1	39.3	38.4
		2	38.2	38.4	38.4	38.7	38.4
		Av.	38.0	38.3	38.5	38.8	38.4
		0.5	50.7	50.9	51.0	52.3	51.2
		1	50.6	51.0	51.3	51.5	51.1
5	50.1	1.5	50.7	51.0	51.0	51.5	51.0
		2	50.8	51.2	51.3	52.4	51.4
		Av.	50.7	51.0	51.1	51.9	51.2
		0.5	62.8	63.1	63.5	63.7	63.3
		1	62.8	63.3	63.8	64.1	63.5
6	62.5	1.5	62.9	63.3	63.4	65.2	63.7
		2	63.1	63.6	63.4	65.4	63.9
		Av.	62.9	63.3	63.5	64.6	63.6
		0.5	75.4	75.9	76.2	77.3	76.2
		1	75.6	75.7	76.5	76.7	76.1
7	75.1	1.5	76.1	76.3	76.8	77.9	76.8
		2	75.5	75.9	76.5	76.9	76.2
		Av.	75.6	76.0	76.5	77.2	76.3
		0.5	99.6	100.0	100.1	101.2	100.2
		1	99.5	99.6	100.7	101.4	100.3
8	100.0	1.5	99.3	100.0	100.1	101.5	100.2
		2	99.4	100.3	100.5	100.5	100.2
		Av.	99.4	100.0	100.4	101.1	100.2
		0.5	45.8	45.9	46.2	46.9	46.2
		1	45.7	45.9	46.3	46.5	46.1
Averages	45.4	1.5	45.8	46.1	46.1	47.0	46.2
		2	45.8	46.1	46.2	46.8	46.2
		Av.	45.8	46.0	46.2	46.8	46.2

the santalenes present were esterified along with the alcohols by the latter method.

With these facts in mind, the following experimental work was performed in an effort to reduce to a minimum the errors in the present procedure, or to find a new and more accurate method of assay.

Experimental Procedure

Samples containing known amounts of free menthol were prepared.

A 1-kg. sample of alpha-pinene was redistilled in vacuum; the major portion of the distillate consisted of a fraction with a constant boiling point of 14° C. at 2 mm. By the regular method of analysis it was found to contain negligible quantities of esters and alcohols. From a 2-kg. lot of natural oil of peppermint the menthol was separated, using the fraction boiling above 70° C. at 2 mm. This crude menthol was purified by centrifuging and by repeated recrystallization from benzene until a constant melting point of 43° C. was obtained for the product. This menthol was dissolved in varying proportions in the redistilled pinene to give samples having definite percentages of free menthol. Using these samples, a time study similar to that of Baldinger (*1*) was conducted.

Pyrex-resistant glassware, with ground-glass joints and interchangeable connections, was used throughout. Electric heaters equipped with sand baths were used and preheated so that samples placed upon them began to boil almost immediately. Acetylation and saponification periods were varied by half-hour intervals up to and including 2 hours. Except for the introduction of these variables, the method described in the U. S. P. XI was employed.

The results of this study (Table I) substantiate the previously mentioned contentions of Baldinger regarding the reaction of alkali during saponification with components in the oil other than esters. The amount of base entering into these side reactions increases sharply if the acetylated oil is permitted to saponify over 1.5 hours. This contributing factor obviously depreciates the classical method by indicating a higher than actual alcohol content.

Several methods (*2*, *3*, *8*), using an acetylant mixture of pyridine and acetic anhydride, have been proposed for the analysis of free primary or secondary alcohols. Because of the objectionable odor of pyridine, an investigation was conducted for solvents that might be substituted in place of this reagent.

Analytical Procedure

One gram of the sample, accurately weighed in a tared acetylation flask, is treated with 5 ml., accurately measured by means of a Koch microburet, of a freshly prepared acetylant mixture consisting of four parts by volume of *n*-butyl ether and one part of acetic anhydride. A blank is prepared in an identical manner, omitting the oil. The flasks are connected to air condensers and the contents boiled gently for 1 hour on a sand bath. Without removing the flasks from the bath, 20 ml. of hot distilled water are added through the condensers and the contents are boiled vigorously for an additional 30 minutes to convert the excess anhydride into acetic acid. After removing the flasks from the bath and allowing the contents to cool to room temperature, 20 ml. of cold distilled water are added as before.

Table II. Free Alcohol Determinations Using Mixtures Containing Known Amounts of Free Menthol

Sample	Calculated (From Table I)	Power and Kleber's Method	Author's Method Mean	Author's Method Average deviation
	%	%	%	%
1	0.0	0.1	0.1	0.1
2	12.6	13.0	12.5	0.2
3	25.5	26.0	25.8	0.3
4	37.5	38.4	37.6	0.5
5	50.1	51.0	50.1	0.2
6	62.5	63.3	62.4	0.3
7	75.1	75.7	75.0	0.5
8	100.0	99.6	99.8	0.4
Av.	45.4	45.9	45.4	0.3

Table III. Analyses of Fractions from a Vacuum Distillation of Oil of Peppermint

Fraction	Distilled	Power and Kleber's Method Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method Mean	Average deviation
	%	%	%	%	%	%
Light fraction	0.8	...	...	...	...	...
Water	0.7	...	...	...	...	...
1	5.0	1.7	4.9	3.6	1.3	0.2
2	5.0	0.9	2.5	1.8	0.6	0.2
3	5.0	1.1	3.0	2.1	1.2	0.5
4	2.5	4.3	19.0	15.6	15.8	0.0
5	10.0	2.3	10.5	8.7	6.9	0.2
6	10.0	1.6	18.0	16.7	15.4	0.2
7	5.0	3.0	43.4	41.1	40.5	0.5
8	5.0	4.2	62.4	59.0	59.4	0.1
9	10.0	5.8	80.4	75.8	75.3	0.2
10	32.5	8.1	91.3	84.9	84.8	0.3
11	2.0	17.2	86.8	73.2	71.2	0.6
Residue	3.5	10.1	27.4	19.4	15.0	0.4
Loss	3.0					
	Av.	5.2	51.9	47.8	46.9	0.3

The flasks are removed from the condensers and the ground-glass connections rinsed by means of a water wash bottle, allowing the rinsings to flow into the flasks. At this point, the acid strength of the blank is approximately 0.5 *N*. Eight or 10 drops of phenolphthalein test solution (dissolve 1 gram of phenolphthalein in 100 ml. of alcohol) are added and the excess acid is neutralized with 0.5 *N* alcoholic potassium hydroxide solution. The blank is titrated to the full red color of the indicator and the oil sample matched with the blank. It is necessary to conduct this titration on a 2-phase system, but this presents no difficulty. The difference in the amount of potassium hydroxide indicates the per-

centage of free alcohols present in the original sample; the calculation of these results is made by the following formula:

$$\% \text{ free alcohol} = \frac{\frac{\text{molecular weight of alcohol}}{20} \times \text{ml. } 0.5\ N \text{ KOH}}{\text{weight of sample}}$$

If the sample to be analyzed is not perfectly anhydrous, it should be dried; allowance should be made for the acid value of those oils containing free acids.

If the percentage of total alcohols is desired, an ester determination is made by the regular method and the following formula will effect the necessary conversion:

$$\% \text{ total alcohols} = \% \text{ free alcohols} + \frac{\text{molecular weight of alcohol}}{\text{molecular weight of ester}} \times \% \text{ esters}$$

Results obtained by the method of Power and Kleber and the procedure outlined above are given in Tables II to VI. To conserve space all figures are averages of several determinations. If the above procedure is carefully followed, the results obtained are very consistent, as indicated by the average deviations from the mean.

The data in Table IV indicate that in the case of oil of peppermint free alcohol determinations by the author's method average about 3 per cent lower than those done by the method of Power and Kleber. In Table III this divergence is traced to small quantities of aldehydes contained in the lower boiling fractions, which aldehydes, it is believed, are acetylated by the pharmacopoeial process, and to polymerization during saponification of the higher boiling resinous constituents, with a subsequent utilization of alkali. The data on redistilled and badly oxidized oil of peppermint indicate that the amount of resinous material affects the extent of the divergence.

The data presented would indicate that a free alcohol content of 43 per cent for oil of peppermint, when analyzed by the method herein described, would very closely approximate the U. S. Pharmacopoeia 50 per cent total alcohol minimum or the British Pharmacopoeia 46 per cent free alcohol minimum if the analysis were made by the classical procedure.

The pure alcohols included in Table VI give harmonious results regardless of the method used, except that *d*-neomenthol must be saponified for more than 3 hours if the U. S. P. XI procedure is used (*5*).

Differences obtained when assaying the impure alcohols included in Table VI or the essential oils shown in Table V are explained by the presence of nonalcohol constituents which are acetylated by the classical method but are not attacked by the acetylant mixture proposed in this paper.

TABLE IV. ANALYSES OF SAMPLES OF PEPPERMINT

Sample	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%
Choice Natural Peppermint					
1	3.7	43.6	40.7	36.4	0.2
2	4.5	47.1	43.6	41.9	0.4
3	5.6	50.0	45.6	42.6	0.3
4	5.2	50.4	46.3	44.5	0.4
5	5.2	51.9	47.8	45.2	0.2
6	5.9	54.6	50.0	47.4	0.2
7	7.5	57.1	51.2	47.7	0.3
8	7.2	59.7	54.0	50.8	0.3
9	8.4	63.9	57.3	53.4	0.4
Av.	5.9	53.1	48.5	45.5	0.3
Redistilled Oil of Peppermint					
1	5.3	52.0	47.8	45.3	0.3
2	5.6	52.8	48.4	45.5	0.2
3	5.5	52.6	48.3	45.6	0.2
4	6.3	54.4	49.5	46.4	0.1
5	5.7	57.0	52.5	50.2	0.5
6	5.8	57.1	52.5	49.8	0.4
Av.	5.7	54.3	49.8	47.1	0.3
Badly Oxidized Oil of Peppermint					
1	9.5	54.8	47.4	43.0	0.3
2	10.7	59.4	51.0	46.8	0.1
3	7.8	58.9	52.7	50.5	0.2
4	11.0	62.9	54.3	52.0	0.8
5	8.5	63.9	57.2	52.7	0.2
6	9.0	63.5	56.4	53.2	0.2
Av.	9.4	60.6	53.2	49.7	0.3

TABLE V. ANALYSES OF OTHER ESSENTIAL OILS

Sample	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%
Citronella	10.1	81.8	73.8	37.3	0.4
Geranium	25.8	69.3	52.5	38.9	0.3
Lavender	41.5	63.2	30.6	14.1	0.2
Rosemary	5.1	19.0	15.0	9.9	0.3
Sandalwood	2.7	92.9	90.6	81.2	0.4

TABLE VI. ANALYSES OF STOCK SAMPLES OF ALCOHOLS

Sample	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%
d-Borneol	0.1	100.1	100.0	99.6	0.2
Cinnamic alcohol[a]	3.6	97.5	94.8	87.7	0.2
Citronellol[a]	4.1	101.8	98.6	91.6	0.3
Geraniol	0.3	94.9	94.7	95.9	0.6
l-Menthol	0.1	99.7	99.6	99.8	0.4
d-Neomenthol[b]	0.7	...	...	98.6	1.2
Phenyl ethyl alcohol	0.3	99.9	99.7	99.4	0.4
Isopulegol[a]	0.5	100.1	99.7	94.2	0.5

[a] No attempt was made to purify these samples. Their aldehyde content was found sufficient to account for differences in results by the two methods.
[b] Sample kindly furnished by Swann and Co., Birmingham, Ala. Hydrolysis of acetylated oil is incomplete in 1-hour saponification limit specified in U. S. P. XI (*5*).

Conclusions

The method of Power and Kleber has been thoroughly investigated. Sources of discrepancies have been found and suggestions made to prevent their occurrence.

A new simplified procedure, utilizing an acetylant mixture of *n*-butyl ether and acetic anhydride, permits a more selective esterification of alcohols than the pharmacopoeial method. This procedure is apparently applicable, without variations, for the analysis of free primary or secondary alcohols, regardless of content, in any of the essential oils. Since saponification, shown to be a major source of error in the method of Power and Kleber, is eliminated, the procedure described gives more satisfactory results with those alcohols whose esters hydrolyze slowly or with difficulty and permits the determination of the alcohol content of those oils whose specific gravities are greater than one. This method is more economical and less time-consuming than that of Power and Kleber. The water present in the titration mixture enables the analyst more easily to distinguish a very sharp end point and prevents the formation of an interfering precipitate.

The procedure described also seems to be more satisfactory than those using a pyridine–acetic anhydride mixture. The odor of *n*-butyl ether is not objectionable and this solvent possesses the added advantage over pyridine that its boiling point (142° C.) coincides well with that of acetic anhydride (140° C.), whereas pyridine boils at 115° C. More rapid and complete acetylation would therefore be indicated in an ether mixture than in a pyridine–acetic anhydride mixture. Greater dilution of the acetic anhydride is possible if *n*-butyl ether is used as a solvent, decreasing the percentage of error in the results.

Acknowledgment

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Methods for Estimation of Some Amino Acids in Corn Grain

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DURING investigations on the chemical composition of inbred and hybrid lines of corn, it was found desirable to determine some of the amino acids present in the corn protein. Similar work on corn has been carried out by Csonka (*3*), Hamilton *et al.* (*8*), and Showalter and Carr (*29*). Detailed investigations on various purified corn proteins have been carried out by numerous workers. The classical work of Osborne and co-workers (*20*, *21*, *22*) as well as the work of Dakin (*4*) and Kossel and Kutcher (*14*) should be recalled in this connection.

The methods used by these workers give very good results but are too long and tedious to be applied to protein studies on large numbers of samples. The work reported here had as its object developing relatively short and accurate methods for the determination of some of the nutritionally important amino acids present in corn grain.

Preparation and Hydrolysis of Protein Fraction

Dry corn grain normally contains 7 to 11 per cent protein, 1 to 2 per cent ash, 3 to 5 per cent fat, 1.5 to 3 per cent crude fiber, and 80 to 85 per cent starch, sugars, and other nitrogen-free materials. In order to obtain the corn protein relatively free from the other constituents of corn grain, it is necessary either to extract the protein or remove the other compounds from the protein.

Hamilton *et al.* (*8*) removed fat and other materials by extraction with dry ethyl ether and absolute ethanol, removed starch by extraction with hot 2 per cent trichloroacetic acid, and then removed the protein by successive treatment with 20 per cent hydrochloric acid and 5 per cent sodium hydroxide.

Csonka (*3*) extracted some of the protein with cold 1 per cent sodium chloride and 80 per cent ethanol. Starch was removed from the residue with cold 21 per cent hydrochloric acid and precipitated from solution with ethanol. The starch-free residue, the acid alcohol solution from the starch precipitation, and the sodium hydroxide and 80 per cent alcohol extracts were hydrolyzed and the hydrolyzate was used as material for the determination of amino acids.

These procedures are both rather long and cumbersome. In an effort to simplify them the following procedure was developed and found satisfactory:

Grind the air-dry corn grain to 1 mm., dry in a vacuum oven at 100° C. for 5 hours, and extract with anhydrous ethyl ether in a continuous Soxhlet extraction apparatus for 48 hours. The ether extract contains only very slight amounts of nitrogen and may be discarded. Grind the dry fat-free residue to 100-mesh in a ball mill. Place 5 grams of the dry finely ground residue in a 200-ml. Erlenmeyer flask, add 150 ml. of distilled water, and heat for 30 minutes on the steam bath to gelatinize the starch. Cool, add 2 ml. of saliva and a few milliliters of toluene, stopper, and incubate at 38° C. for 72 hours with occasional shaking. At the end of the incubation period, heat on the steam bath to inactivate the enzyme and drive off the toluene. Filter through Whatman No. 42 filter paper and wash thoroughly with hot water. Discard the filtrate.

Transfer the residue to a 250-ml. Kjeldahl flask and add 20 ml. of hydrochloric acid, specific gravity 1.10. Add a few drops of capryl alcohol, place a test tube through which water is flowing in the neck of the flask to act as a reflux, and boil gently on a sand bath for 20 hours. Remove the condenser and boil off the capryl alcohol. Filter off the humin and wash thoroughly, catching the filtrate and washings in a 250-ml. Claisen flask. Evaporate the filtrate and washings to a thick paste on a boiling water bath under reduced pressure, keeping the temperature below 60° C. Add 50 ml. of distilled water and again evaporate to a thick paste. Repeat twice more to remove as much of the hydrochloric acid as possible. Add 25 ml. of water to the paste and make just alkaline to litmus with solid calcium hydroxide. Add 25 ml. of ethanol to prevent foaming and distill off the ammonia into an excess of 0.1 *N* hydrochloric acid. Thirty minutes under reduced pressure on a boiling water bath is sufficient to remove the ammonia.

Back-titrate the solution in the receiving flask with 0.1 *N* sodium hydroxide and calculate the amount of ammonia nitrogen present. (Run a blank on the reagents used and deduct this from the value obtained.) Filter off the material remaining in the Claisen and wash with hot water until free from chlorides. Acidify the filtrate and washings with hydrochloric acid and concentrate under reduced pressure to about 10 ml. Transfer to a 50-ml. volumetric flask and make to volume with distilled water. Pour into a 150-ml. beaker, add a few grams of Norit A (which has been washed with acid and alkali), stir thoroughly, and filter. This treatment was found to decolorize the solution satisfactorily. Determine cystine and cysteine on aliquots of this solution according to the directions given below.

Pipet another aliquot (25 ml.) of the solution into a 50-ml. centrifuge tube and precipitate the basic amino acids with phosphotungstic acid according to the directions given by Cavett (*2*). When washing the phosphotungstic acid precipitate, keep the wash solution, the centrifuge tubes, and the tube holders as nearly ice cold as possible. To the washed phosphotungstic acid precipitate add a thick suspension of barium hydroxide with stirring until the solution is just alkaline to litmus. Centrifuge off the barium phosphotungstate and wash several times by dispersing the precipitate in hot distilled water and centrifuging. Combine the filtrate and washings and precipitate the excess barium by the addition of a slight excess of sulfuric acid. Filter off the barium sulfate and wash with hot distilled water. Concentrate the filtrate and washings to a small volume under reduced pressure, transfer to a 25-ml. flask, and make to volume. Use aliquots of the solution for the determination of arginine and histidine according to the directions given below.

For the determination of tyrosine and tryptophan another sample of corn is hydrolyzed with alkali, since tryptophan is partially destroyed by acid hydrolysis in the presence of carbohydrate material (*9*).

Transfer the residue from the saliva digestion of 5 grams of dry, fat-free corn to a test tube and add 20 ml. of 5 *N* sodium hydroxide. Insert an air condenser (a glass tube about 30 cm. long through a stopper covered with tin foil) and digest for 24 hours in the steam bath. Wash the hydrolyzate into a 50-ml. volumetric flask with water. Add 10 ml. of 15 *N* sulfuric acid

TABLE I. NITROGEN DISTRIBUTION OF FRACTIONS FROM A SAMPLE OF DRY, FAT-FREE CORN GRAIN

Fraction	Equivalent Weight of Corn	Nitrogen	% of Total Nitrogen in Fat-Free Corn
	Grams	*Mg.*	
Dry, fat-free corn grain	1	17.4	100.00
Filtrate from saliva digestion	2	2.80	8.04
Acid-insoluble humin	5	3.12	3.59
Acid-soluble humin	5	1.26	1.45
Ammonia nitrogen	5	11.07	12.73
Acid hydrolyzate	1	12.58	72.30

mix thoroughly, cool, and make to volume. Filter or centrifuge the solution. Decolorize the filtrate with Norit A (previously washed with acid and alkali) and use an aliquot for the determination of tryptophan and another aliquot for the determination of tyrosine according to the procedure given below.

For tryptophan a longer hydrolysis period may be necessary or the determination may be carried out on the unhydrolyzed residue by the original May and Rose procedure (*19*).

These hydrolysis procedures are not new. The acid hydrolysis is essentially that described by Cavett (*2*), while the alkaline hydrolysis is similar to that described by Folin and Marenzi (*6*) and Lugg (*18*). The author is not aware of any previous use of saliva to remove starch in protein purification. Some nitrogen compounds, the nature of which is not known, are lost in the filtrate from the saliva digestion. Acetic acid, trichloroacetic acid, and basic lead acetate do not cause the precipitation of any nitrogen-containing compounds from this filtrate, indicating that the nitrogen is not protein nitrogen. The solution gives only a faint ninhydrin reaction, which indicates that only small amounts of proteins or amino acids are present.

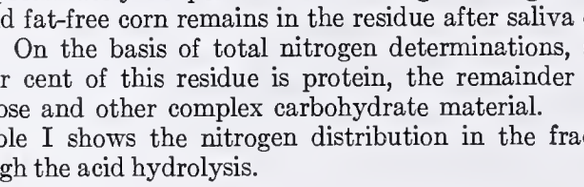

FIGURE 1. PURE CYSTINE SOLUTIONS

Approximately 20 per cent of the original weight of the ground fat-free corn remains in the residue after saliva digestion. On the basis of total nitrogen determinations, about 45 per cent of this residue is protein, the remainder being cellulose and other complex carbohydrate material.

Table I shows the nitrogen distribution in the fractions through the acid hydrolysis.

Colorimetric Determination of Amino Acids

Colorimetric methods for the determination of some of the amino acids have been carefully studied and shown to give as accurate results as direct isolation methods. In general colorimetric methods are less cumbersome and time-consuming than direct isolation or gravimetric methods. Colorimetric methods may often be simplified and improved by using some type of photometer, so that reference data can be obtained from pure solutions of the substance to be determined. These reference data may then be used in the analysis of unknown samples, thus obviating the necessity of carrying standard solutions through the procedure with each set of unknowns.

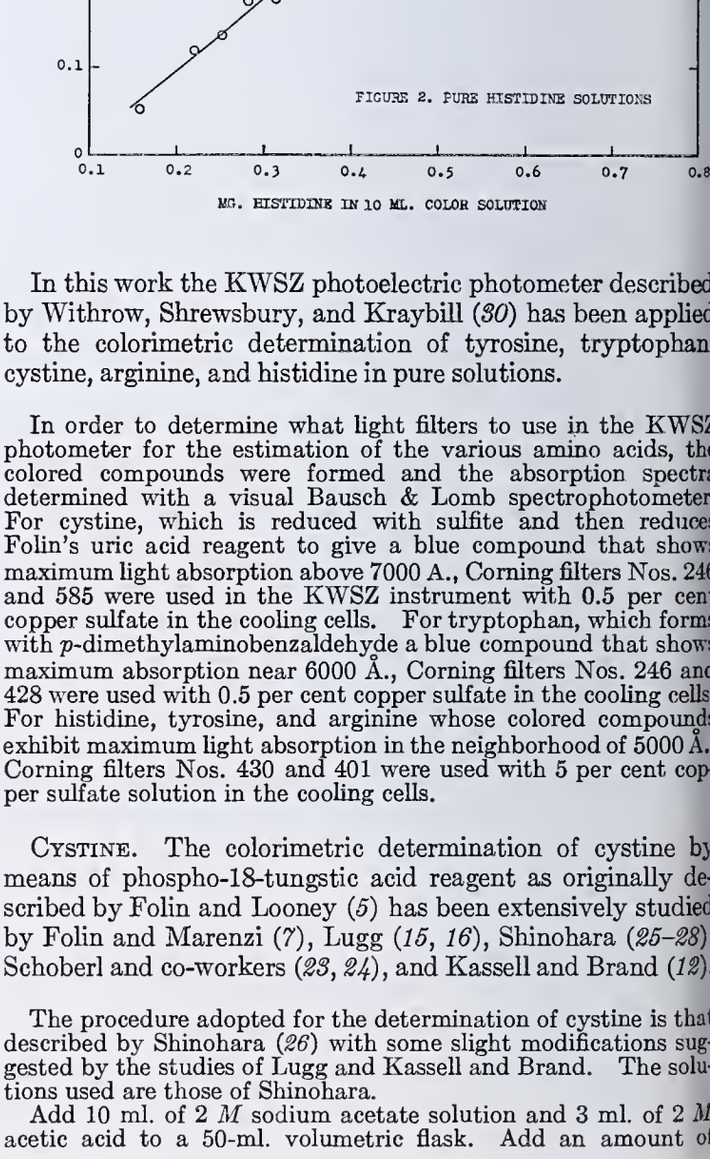

FIGURE 2. PURE HISTIDINE SOLUTIONS

In this work the KWSZ photoelectric photometer described by Withrow, Shrewsbury, and Kraybill (*30*) has been applied to the colorimetric determination of tyrosine, tryptophan, cystine, arginine, and histidine in pure solutions.

In order to determine what light filters to use in the KWSZ photometer for the estimation of the various amino acids, the colored compounds were formed and the absorption spectra determined with a visual Bausch & Lomb spectrophotometer. For cystine, which is reduced with sulfite and then reduces Folin's uric acid reagent to give a blue compound that shows maximum light absorption above 7000 A., Corning filters Nos. 240 and 585 were used in the KWSZ instrument with 0.5 per cent copper sulfate in the cooling cells. For tryptophan, which forms with *p*-dimethylaminobenzaldehyde a blue compound that shows maximum absorption near 6000 Å., Corning filters Nos. 246 and 428 were used with 0.5 per cent copper sulfate in the cooling cells. For histidine, tyrosine, and arginine whose colored compounds exhibit maximum light absorption in the neighborhood of 5000 Å., Corning filters Nos. 430 and 401 were used with 5 per cent copper sulfate solution in the cooling cells.

CYSTINE. The colorimetric determination of cystine by means of phospho-18-tungstic acid reagent as originally described by Folin and Looney (*5*) has been extensively studied by Folin and Marenzi (*7*), Lugg (*15*, *16*), Shinohara (*25–28*), Schoberl and co-workers (*23*, *24*), and Kassell and Brand (*12*).

The procedure adopted for the determination of cystine is that described by Shinohara (*26*) with some slight modifications suggested by the studies of Lugg and Kassell and Brand. The solutions used are those of Shinohara.

Add 10 ml. of 2 *M* sodium acetate solution and 3 ml. of 2 *M* acetic acid to a 50-ml. volumetric flask. Add an amount of

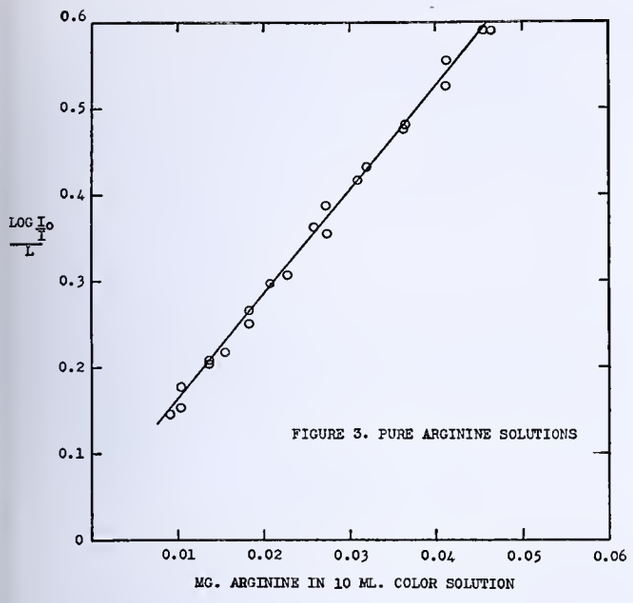

FIGURE 3. PURE ARGININE SOLUTIONS

ution containing 0.2 to 1.0 mg. of cystine, enough sodium droxide solution to neutralize the acidity of the added cystine ution (determined by titration of a separate aliquot of the ution using bromophenol blue as the indicator), and 2 ml. of *M* sodium bisulfite solution. Place in a water bath at 18° ± C. for 15 minutes, add 2 ml. of phosphotungstic acid reagent, x, and allow to stand in the water bath for 15 minutes. Dilute volume with water and after 5 to 10 minutes determine the ht transmission in the KWSZ photometer, with water in the erence cell. Since the sulfite itself develops a small amount of or with the phosphotungstic acid reagent, it is necessary to luct the value obtained from a sulfite blank determination.

Figure 1 shows the curve obtained with pure cystine solu-ns. It will be noted that the log values for the light ab-ption are directly proportional to the cystine concentra-n within the concentration limits used.

Since cystine with sulfite and cysteine with sulfite develop e same amount of color (mole for mole) and cysteine de-lops half as much color in the absence of sulfite as in the esence of sulfite, both cystine and cysteine may be deter-ned using this curve. For this the solutions are those as tlined by Kassell and Brand (*12*).

Histidine. For the quantitative determination of histi-ne the following procedure was found satisfactory:

To a few milliliters of histidine solution (containing 0.15 to 5 mg. of histidine) in each of three 10-ml. volumetric flasks d bromine solution (1.0 per cent bromine in 33 per cent acetic d) dropwise until a deep yellow color persists. Let the solu-ns stand at room temperature for 10 minutes. Remove the ess bromine by bubbling air through the solution until the low color disappears. Add 2 ml. of a solution containing 2 ts of concentrated ammonium hydroxide and 1 part of 10 cent ammonium carbonate to each flask and immerse the ks in a boiling water bath for exactly 5 minutes. Remove the ks from the water bath and immerse in an ice bath for 5 nutes. Allow to stand at room temperature for 10 to 20 nutes, dilute to the mark with 95 per cent ethyl alcohol, and x. Pour the contents of the three flasks into a small Erlen-yer flask, mix, and measure the transmission value in the VSZ photometer within 15 minutes. The mixing of three arate dilutions offers a rapid method for obtaining an average nsmission value from three separate determinations. The nk solution used in the reference cell of the instrument is de up and treated as above, except that water instead of a tidine solution is used.

This is an adaptation of the Wooley and Peterson modifica-n (*31*) of the Kapeller-Adler procedure (*11*) for the determina-n of histidine.

Figure 2 shows the curve obtained using pure histidine solu-ns. The log values for the light absorption are propor-nal to concentration when the histidine concentration is between 0.45 and 0.80 mg. of histidine in 10 ml. of color solution.

Arginine. Arginine was determined as follows:

Place an amount of arginine solution containing 0.01 to 0.05 mg. of arginine in each of four 10-ml. volumetric flasks and add water to make 5 ml. Cool in an ice bath, add 1 ml. of 10 per cent sodium hydroxide solution and 1 ml. of 0.02 per cent α-naphthol, mix, and embed in ice for 1 hour. Add 0.1 ml. of sodium hypobromite solution (2.5 grams of bromine dissolved in 100 ml. of cold 5 per cent sodium hydroxide), shake vigorously, and exactly 15 seconds later dilute to volume with cold 40 per cent urea solution. Pour the solutions from the four volumetric flasks into an Erlenmeyer flask, mix, and measure the transmission in the KWSZ photometer after 5 minutes and in less than 7 minutes after adding the hypobromite, with water in the reference cell.

Four dilutions are made and the four solutions mixed before reading, because even pure solutions of arginine do not always develop the same shade of color under the conditions outlined. The mixing of four separate dilutions is a rapid method for determining the average transmission value of four separate dilutions.

This is an adaptation of the procedure given by Jorpes and Thoren (*10*) for the determination of arginine by the Sakaguchi reaction.

Figure 3 shows the curve obtained with pure solutions of arginine. Within the concentration range used the log values for the light absorption are proportional to the arginine concentration and Beer's law is obeyed.

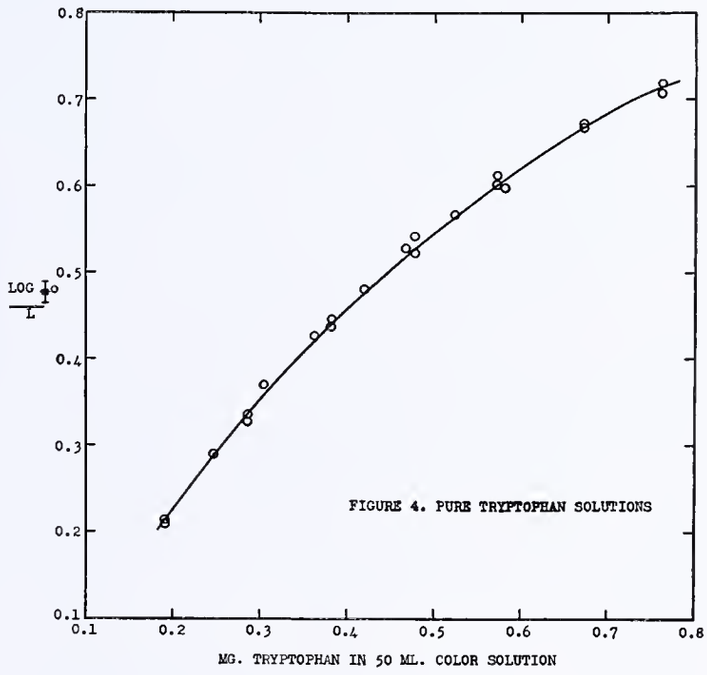

FIGURE 4. PURE TRYPTOPHAN SOLUTIONS

Tryptophan. The following procedure was used for the colorimetric determination of tryptophan:

Pipet an amount of tryptophan solution containing 0.2 to 0.8 mg. of tryptophan into a 50-ml. volumetric flask. Add 2 ml. of 1 per cent tryptophan-free gelatin solution, 0.2 ml. of 1.5 per cent sodium nitrate solution, 0.5 ml. of 5 per cent *p*-dimethylaminobenzaldehyde solution, and 2.5 ml. of concentrated hydrochloric acid. Mix and allow to stand for 20 minutes at room temperature. Dilute to volume with water, mix, and immerse in an ice bath for 30 minutes. Subject the solution to colorimetry in the KWSZ photometer. The blank solution used in the reference cell should be made up and treated as above, using water instead of a tryptophan solution.

This is an adaptation of methods described by Bates (*1*) and Komm (*13*) who modified the original May and Rose (*19*) procedure.

Figure 4 shows graphically the data obtained with pure tryptophan solutions. The log values for light absorption are not proportional to tryptophan concentration when the color is developed according to the above procedure. How-

TABLE II. DATA ON METHODS

Amino Acid	Cystine	Histidine	Arginine	Tryptophan	Tyrosine
Reagent used	Phospho-18-tungstic acid	Bromine and NH_4OH	α-Naphthol and sodium hypobromite	*p*-Dimethylamino-benzaldehyde	Millon's reagent
Filter system, Corning filters, No.	246 and 585	430 and 401	430 and 401	246 and 428	430 and 401
Heat filter cell, copper sulfate, %	0.5	5	5	0.5	5
Is concentration proportional to light absorption?	Yes	Yes, between 0.45 and 0.8 mg.	Yes	No	Yes
Concentration range, mg.	0.2 to 0.9 in 50 ml.	0.2 to 0.8 in 10 ml.	0.01 to 0.05 in 10 ml.	0.2 to 0.75 in 50 ml.	0.25 to 0.9 in 25 ml.
Time limit on reading, min.	5 to 10	15	5 to 7	30 to 40	5 to 10
Color of solution	Blue	Blue-violet	Orange	Blue	Red orange
Accuracy, %	±3	±4	±5	±3	±1.5

ever, the method is sensitive for small amounts of tryptophan and with pure solutions gives results that are accurate within ±3 per cent.

TYROSINE. The color developed by Millon's reagent was used for the determination of tyrosine.

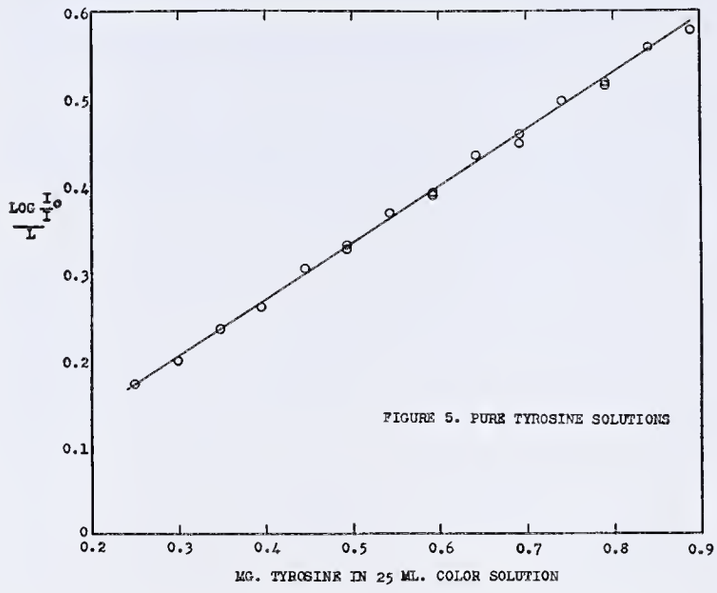

FIGURE 5. PURE TYROSINE SOLUTIONS

The procedure is an adaptation of the methods described by Folin and Marenzi (*6*) and Lugg (*17*). The reagents are those described by Lugg.

To a few milliliters of tyrosine solution (containing 0.25 to 0.9 mg. of tyrosine) in a 25-ml. volumetric flask add enough water to make 3.5 ml. and 1.5 ml. of 5 *N* sulfuric acid. Add 5 ml. of the mercury salt–sulfuric acid solution described by Lugg, mix, and maintain at 60° to 65° C. for 30 minutes. Cool in tap water and allow to stand at 18° to 20° C. for 10 minutes. Add dilute mercury salt–sulfuric acid solution nearly to the mark and mix. Add 0.1 ml. of 1 *M* sodium nitrite solution, make to volume with dilute mercury salt–sulfuric acid solution, and mix. After 5 minutes and in less than 10 minutes measure the transmission in the KWSZ photometer. In the reference cell use a solution prepared in an identical manner except that water replaces the tyrosine solution. If tryptophan is present in the unknown solution, the mercury salt of tryptophan must be centrifuged out and washed as described by Lugg, and the solution must stand at 18° to 20° C. for 1 hour instead of only 10 minutes as recommended above.

Figure 5 shows the curve obtained using pure tyrosine solutions. The log values of light absorption are proportional to tyrosine concentration and Beer's law is obeyed.

The methods outlined above may be applied to the determination of the amino acids in the solutions obtained by the procedure outlined in the early part of this paper. The curves obtained with pure solutions of the amino acids are used as reference data for the determination of the amino acids in the unknown solutions.

The data obtained with the KWSZ photoelectric photometer using pure solutions of the amino acids show that colorimetric determinations can be made with the following accuracy: tryptophan ±3, histidine ±4, tyrosine ±1.5, arginine ±5, and cystine ±3 per cent. The actual error in the transmission determination by the photometer is much less than 1 per cent; additional inaccuracy enters because of difficulties encountered in the development of colored compounds with the various amino acids.

Summary

Table II shows some of the important points concerning the methods used.

The KWSZ photoelectric photometer has been applied to the colorimetric determination of tyrosine, tryptophan, arginine, histidine, and cystine in pure solutions.

The data obtained from pure solutions of these amino acids are being used as reference data in the determination of the amino acid content of corn grain. Starch is removed from the dry fat-free corn by digestion with saliva and the residue hydrolyzed with acid. Cystine is determined in an aliquot of the acid hydrolyzate. Histidine and arginine are determined in a solution from the phosphotungstic acid precipitate of an aliquot of the acid hydrolyzate. Tyrosine and tryptophan are determined in an alkali hydrolyzate of the residue after removal of starch by saliva digestion.

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New Instrument for Rheological Studies of Plastic Substances

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In this paper are discussed the aims, details of construction, accessories, and procedure of a new instrument for rheological studies of plastic substances. The instrument is to be regarded not as a fixed quantity invariable in make-up but as an embodiment of principles for accomplishing a certain purpose—namely, study of mechanical properties observable during deformation of plastic substances.

Suitably modified forms of the instrument and its accessories should be useful in both control and research related to pectins, jellies, gelatins, oil-well drilling muds, ceramic slips, pharmaceutical gels, thermoplastics, and polymerplastics. In fact, the principles may be employed in studying any materials which may be allowed to set under conditions of controlled past history (or path) with a view to measuring the forces required to produce incipient flow or permanent deformation. However, in this paper the instrument is discussed from the point of view of its use as a research tool in studying lyogels.

IF A SOLID substance exists in such a state that it can undergo permanent deformation without fracture, it is said to be a plastic substance (*6*). Because of the increasing importance of such substances in several industries, it has become important to be able to measure some intensive property which would be characteristic of the state of a plastic material. This implies that the property chosen must be reproducibly detectable—for example, by obvious permanent distortion. Concepts based on detectable permanent distortion are proportional limit, yield points, and ultimate strength. These three properties are usually determined for defining the strength of a material (*7*).

Proportional limit has been defined (*5*) as the stress at which the deformation ceases to be proportional to the load; yield point (*5*) as the stress at which marked increase in deformation of specimen occurs without increase in load; and ultimate strength (*8*) as the stress obtained by dividing the maximum load reached, before breaking the specimen, by the initial cross-sectional area, thus giving the dimensions of force divided by length squared. This quantity is often taken as a basis for determining working stresses, and is sometimes used as a characteristic property of a material. If the force applied before reaching the ultimate strength is plotted against displacement, the area under the curve represents work done in producing structural breakdown. It is the purpose of this article to describe an instrument which makes possible the quantitative study of these characteristics of plastic materials.

The instrument has been used in studying the forces required to produce breakage of certain bentonite lyogels. Results and theoretical conclusions resulting from these studies are to be found elsewhere (*2*).

The instrument embodies the torsion principle which has been used previously in certain viscometers and by Garrison (*4*) in his torsion wire gelometer. However, in the instrument described here, the sample is actually sheared and measurement is made of ultimate strength within the sample rather than of forces between interfaces.

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Details of Construction

ESSENTIAL MECHANICAL PARTS. Figure 1 represents a cross section from the front to the rear through the central vertical axis of the instrument. Some of the parts have been overproportioned, so that all the significant details may be shown on one cross-sectional view. Rigid support of the essential parts of the instrument is obtained by mounting them on platforms which slide up and down the vertical race, *RA*, of a floor-type drill stand and may be fixed at any vertical position by tightening the clamp handles, C_1, C_2, C_3.

It seems best to regard the instrument as built around the sample to be tested, which occupies the position designated as *S*. The sample at *S* is supported in a circular trough on a glass disk, *SD*, 4 inches in diameter and 0.25 inch thick (see also Figures 2, 3, and 4). The vertical walls of the circular trough are made of sections cut from beakers and set in concentric grooves on the disk as follows: A circular concentric groove is cut in the top of the disk at a radius of 1.33 inches to a depth of 0.125 inch. Hermetically sealed in this groove is a section, *SB*, cut from a beaker, which serves as the outside container wall. The section of beaker is 2.7 inches in outside diameter, 1.4 inches deep, and has a wall thickness of 0.05 inch. Twelve equally spaced Pyrex fins, *F*, are cemented by means of de Khotinsky cement.

Figure 4 shows an assembly having an outside wall only. The inside wall of the circular trough to contain the sample as shown in Figure 1 is likewise cut from a beaker 0.9 inch in outside diameter and 1.25 inches deep and has a wall thickness of 0.05 inch. It also has Pyrex fins cemented vertically to the outside surface of its walls. These vertical fins on both walls prevent the sample from slipping at the wall interface and thus cause the shearing force to be applied in the limited vertical cylindrical path of the prongs. The disk supporting the sample and forming the bottom of its container is supported inside a water jacket, *J*, made of concentric brass tubing 4 and 4.5 inches in diameter and 18 inches long. This water jacket is movable vertically and is freed or fixed by loosening or tightening clamp handle C_1.

Suspended from above and dipping into the sample are six prongs, *PR*, two of which are shown in cross section. These Pyrex glass rods are 0.2 inch in diameter and 1.7 inches long. They are supported through a glass disk, *D*, of 2.75 inch outside diameter, 0.65 inch inside diameter, and 0.25 inch thickness, drilled with six holes having 0.2 inch diameter equally spaced in a concentric circle of 1.43 inch radius. The prongs are sealed in the six equally spaced holes by means of a sodium silicate cement. Figure 4 shows this disk-prong assembly.

A rigid pointer extends horizontally across the diameter of the top of the glass disk, to indicate the position of the disk as regards rotation. The disk is in turn supported through a grooved rubber gasket tightened by two nuts on a hollow, threaded, cylindrical brass rod having outside diameter of 0.5 inch and a right circular conical segment hole through its center. By means of a six thirty-two screw, 0.5 inch long, this fitting is tightly pressed onto a pinion, *P*, made up of brass in one piece having cylindrical upper and right circular conical lower section. The cylindrical shoulder section is 0.4 inch in diameter and 0.45 inch in length, and the frustrum of right circular conical segment is 0.85 inch long, 0.325 inch in diameter at top, and 0.25 inch in diameter at bottom. This pinion is soldered to the lower end of the torsion wire (piano wire), *TW*, which supports the disk-prong assembly. The wire is 11.4 inches long between fittings.

The upper end of the torsion wire is soldered into a section of a circular brass rod, *R*, 0.25 inch in diameter and 0.25 inch long. Also soldered into *R* is a rigid indicator rod, *I*, made of cold rolled steel 0.125 inch in diameter, 11.2 inches in vertical length, and 2.25 inch in horizontal length at its lower end. The purpose of this pointer is to register in the horizontal plane just above the gel the position of the top of the torsion wire. Torque and deformation in the sample are found by referring the indicating pointer,

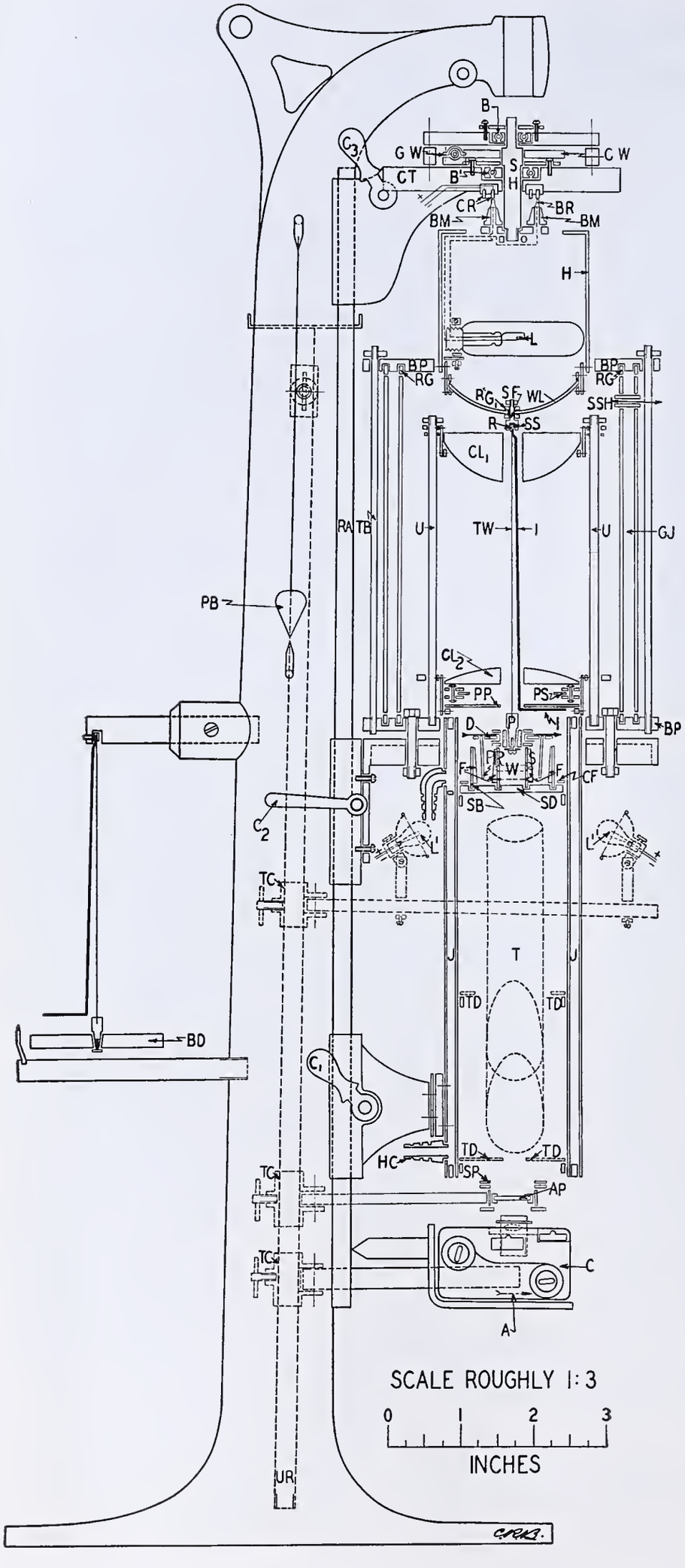

FIGURE 1. VERTICAL CROSS SECTION, SHOWING DETAILS OF CONSTRUCTION

A. Image on film
AP. Analyzer polarizer
B, B'. Ball bearings
BD. Brass disk
BM. Mounts
BP. Brass plates
BR. Brushes
C. Camera
C_1, C_2, C_3. Clamp handles
CF. Circular filters for equalizing actinic value of light through and around sample
CL_1, CL_2. Condenser lenses
CR. Brass rings
CT. Supporting platform
CW. Cogwheel
D. Disk
F. Pyrex fins
GJ. Glass water jacket
GW. Worm gear
H. Housing
HC. Hose connection in lower water jacket
I. Pointer
J. Water jacket
L, L'. Light sources
P. Pinion
PB. Plumb bob
PP. Polarizer disk
PR. Prongs
PS. Protractor scale
RA. Vertical race
R. Rod fitting to support torsion wire and pointer
RG, RG_1. Rubber gaskets
S. Sample
SB. Section of beaker
SD. Glass disk
SF. Fitting
SH. Shaft
SP. Protractor scale referring to analyzer polaroid
SS. Setscrew
SSH. Hole in jacket
T. Tube
TB. Through-bolts
TC. Tee clamps
TD. Tin disks
TW. Torsion wire
U. Brass uprights
UR. Upright rod
W. Stop watch
WL. Watch-glass lens

I, and the pointer on the glass disk-prong assembly to the transparent circular protractor scale, *PS*, which is graduated in percentage degrees and has 4 inch outside diameter and 3.15 inch inside diameter. Below this scale is a polarizer disk, *PP*, having 6 inch outside diameter and 1 inch inside diameter.

The tops of the torsion wire and pointer are supported through the fitting, *R*, by a six thirty-two setscrew, *SS*, in the supporting fitting, *SF*. This fitting passes through a grooved rubber gasket, RG_1, set in a 0.5-inch hole drilled through a watch-glass lens, *WL*, having outside diameter of 5.25 inches, convex radius of curvature 3.5 inches, and thickness of 0.125 inch. This lens is adapted by means of sections of brass tubing to the housing, *H*, also made from brass tubing having diameter of 6.25 inches and length of 6.75 inches. The top of the housing is made from a brass disk 0.125 inch thick and is drilled for ventilation. This housing is held tightly against the shaft, *SH*, by means of a lock nut.

The shaft is supported through the inner races of the ball bearings, *B* and *B'*, having inside diameter of 0.4 and 0.5 inch, respectively, *B'* taking the vertical thrust. The shaft (and thus the entire assembly) may be rotated in the horizontal plane by turning a crank handle which rotates the worm gear, *GW*, 0.6 inch in diameter, 0.8 inch long, 8 threads per inch supported on an axle through two pillow blocks. This gear drives the cog wheel, *CW*, 4.25 inches in diameter, 100 cogs, which is fixed rigidly to the shaft, *SH*.

OPTICAL SYSTEM. To provide means of reading torque displacement and time accurately, an optical system is placed in the same vertical axis as the torsion wire. It is essentially the same as a lantern slide projec-

FIGURE 2. PRONGS RIGIDLY HELD IN DISK SUSPENDED ON TORSION WIRE AND FREE TO ROTATE IN SAMPLE CONTAINER

tion system in which the pointers, transparent circular protractor scale, and sample act as a slide. The system is composed of a 6-volt projection lamp "point" source of light, *L*, mounted in the optical axis but free to rotate with the housing, *H*. This point source of light is supplied with electrical energy through two concentric brass rings, *CR*, embedded in Bakelite. This energy is received by phosphor-bronze brushes, *BR*, supported on porcelain mounts, *BM*. Below this source of light is a watch-glass lens, *WL;* 6-inch diameter, 6-inch focal length plano-convex condenser lens, CL_1, drilled with a 0.5-inch hole through the center; 6-inch diameter, 18-inch focal length, plano-convex lens, CL_2, drilled with 1-inch hole through center; plane polarizer, *PP;* and the object to be projected, consisting of the circular protractor scale, pointers, and sample.

The converging light from CL_2 passes through a plane analyzer polarizer, *AP*, whose plane of polarization can be recorded with reference to the circular protractor scale, *SP*. Then the light converges into the f.3.5, 50-mm. lens of a Sept movie camera. This lens causes the formation of an image on the film in the position of the dotted arrow, *A*. The recording camera also looks through the side tube, *T*, and views the stop watch, *W*, which is in about the same horizontal plane as the gel and is illuminated by the lights, *L'*. Actinic value of the light reflected from the watch through the side tube, *T*, can be regulated by rotating a plane polarizer placed in the upper end of the tube. To prevent glare from the sides of the tube and water jacket, tin disks, *TD*, have been supported on brass springs inside the tubes.

FURTHER DETAILS OF CONSTRUCTION. The lenses, CL_1 and CL_2, and transparent circular protractor and polaroid are supported in short sections of aluminum tubing 6.125 inches in inside diameter by means of a circular spring made from 0.25-inch cold-rolled steel bar. These tube sections are supported by three rigid brass rod uprights, *U*, 13.25 inches long and 0.475 inch in diameter. The plane surface of CL_1 is 10.625 inches above that of CL_2, which is 17.5 inches above the lens of the camera.

Surrounding the 6-inch condenser lens system is a glass water jacket, *GJ*, made from sections of Pyrex tubing 10.1 and 8.75 inches in outside diameter, respectively, 0.25 inch in wall thickness, and 16.25 inches long. These tubes are set into rubber gaskets, *RG*, which are set into grooves in brass plates, *BP*, 0.5 inch thick and 10.75 inches square. These header plates are tightened against the ends of the glass tubing by means of long brass through-bolts, *TB*. A hole through the jacket, *SSH*, 0.5 inch in diameter, has rubber tubing cemented in, so that a long slender screwdriver may be inserted for loosening or tightening the setscrew, *SS*. Hose connections for circulating water through this jacket are not shown; however, they are shown, *HC*, in the lower water jacket. A baffle inside the lower jacket causes the water to circulate around the gel.

A vertical upright rod, *UR*, is seen in dotted lines on the opposite side of the drill stand. This rod is 0.75 inch in diameter and 4 feet 2 inches long. The lights, *L'*, polarizer, *AP*, and camera are supported from it by means of tee clamps, *TC*, which permit these accessories to be swung aside so that the jacket sample container, *J*, may be slipped down on the race, *RA*. With the jacket slipped entirely off the end of the race, the torsion wire, prong, and pointer assembly may be removed or inserted as a unit by holding the rigid pointer, *I*, and loosening or tightening the setscrew, *SS*, by means of a screwdriver through the hole, *SSH*. The sample, after being placed in its container jacket, may be pushed up into position and locked there by means of the clamp handle, C_1.

ACCESSORIES FOR USE IN GELATION STUDIES. An accessory shown on the back of the apparatus is a means of suspending a brass disk, *BD*, of known moment of inertia, on the end of the torsion wire to be calibrated. By the torsion pendulum principle (*3*) the coefficient of torsional stiffness of the wire may be found, thus providing a means of calculating torque from any measured angle of deflection.

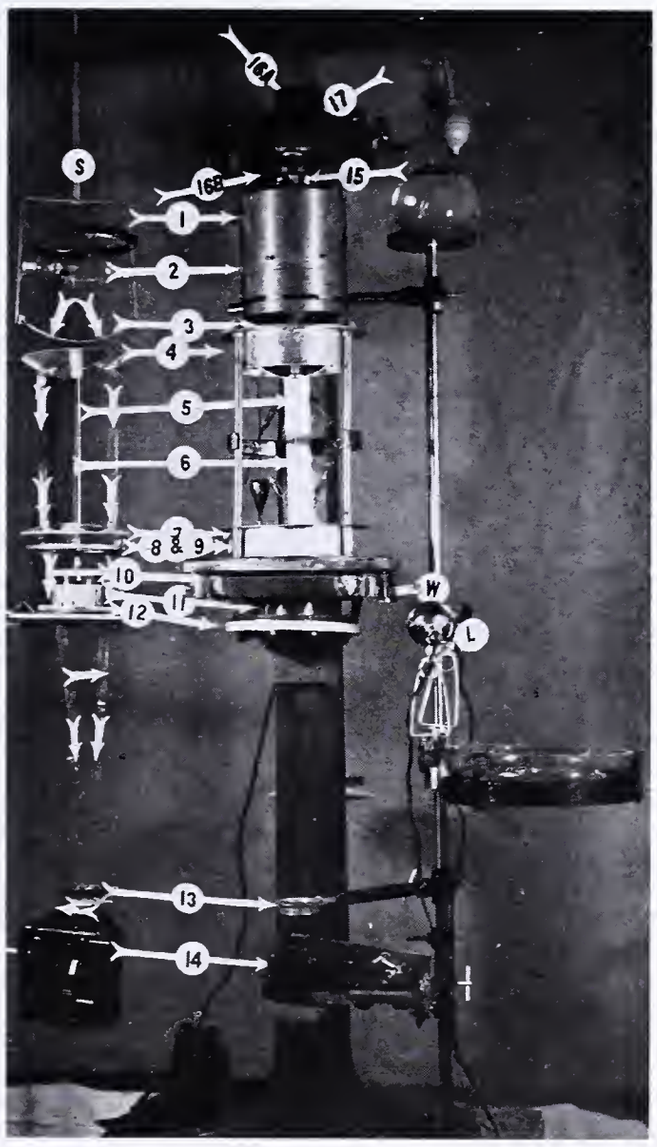

FIGURE 3. ESSENTIAL PARTS OF OPTICAL AND MECHANICAL SYSTEMS

1. Lamp housing
2. Projection lamp bulb
3. Watch-glass lens
4. Condenser lens
5. Pointer indicating position of top of torsion wire
6. Torsion wire
7. Condenser lens
8, 9. Circular protractor and polaroid
10. Disk-prong assembly
11. Wall of sample container
12. Bottom of sample container
13. Analyzer polaroid
14. Camera
15. Phosphor-bronze brush mountings
16*A*. Shaft supporting lamp housing
16*B*. Concentric slip rings for electricity to light filament
17. Gear on shaft for rotating lamp housing
L. Light source
S. Shaft
W. Stop watch

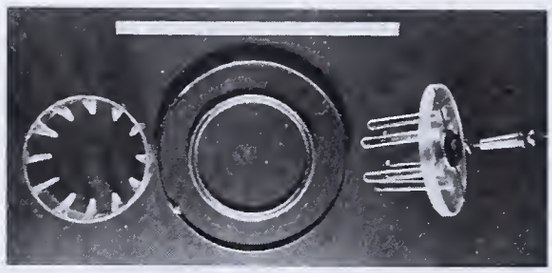

FIGURE 4. PARTS OF SAMPLE CONTAINER AND DISK-PRONG ASSEMBLY

A plumb bob, *PB*, provides a means of leveling the instrument.

Other accessories are shown in Figures 5 and 6. The pump, 16, is used to circulate the water from the constant-temperature tank, 11, through the water jackets. Although the pump is shown in this figure, it was actually isolated from the instrument by using a surge tank to prevent vibration in the water jackets. To prevent mechanical vibration of the floor the pumping mechanism was suspended in a network of rubber tubing.

Submerged in the water in tank 11 is a 2-gallon bottle, the bottom of which has been cut off. In its neck is a rubber stopper in which is inserted a brass tube which makes an arch above and to the right of the tank and enters the water jacket of the instrument. Surrounding this brass tube is a rubber hose. Circulating water passes between the hose and tube. Dry air is blown through a sparger beneath the bottomless bottle in tank 11, becomes saturated by passing up through the water inside, then flows isothermally through the brass tube into the jacket containing the gel sample, thus preventing evaporation while the gel is setting.

Also shown in Figure 5 are a battery, *B*, and transformer, 12, which serve as sources of electrical energy for the lights. An electromagnet for operating the shutter is shown mounted beside the camera.

Consider that a test on a gel has been completed in the instrument. The camera, 6, the lights, *L*, and polarizer, 5, may be swung to the right and the jacket, 7, removed by loosening the clamp handle, 14, and slipping the jacket support down off the race. The dish, 9, is then swung under the prongs, which may be cleansed using the wash bottle, 10, and the brush, 4, the washings dropping into the dish.

Procedure for Use

With reference to Figure 5, consider that the lights, *L*, polaroid, 5, and camera, 6, have been swung to the right and the water jacket, 7, has been lowered off the end of the race as described above. The torsion wire assembly may now be inserted from below through the holes in the lenses into the fitting, *SF* (Figure 1). The setscrew, *SS*, is now tightened by means of a long screwdriver inserted through the opening, 13, in the glass water jacket. The gel to be tested is placed in its container in the brass water jacket, 7, which is then slipped back and clamped in position, so that the prongs suspended from the torsion wire dip into the specimen to the proper depth as indicated by a pointer on the clamp with reference to a scale marked on the race, *RA* (Figure 1). All openings in the jackets are then closed as tightly as possible, and humid air is admitted during gelation. While the gel is setting, isothermal conditions are maintained by circulating water in the jackets.

At the beginning of the test the lights are turned on and a picture is made to record relative zero readings of the pointers, showing the positions of the top and bottom of the torsion wire with reference to the transparent protractor scale. The crank, 17, is then turned, driving the worm gear which causes the top of the torsion wire to rotate. Rotation of the bottom of the torsion wire is restrained by the sample up to a maximum torque at which there is suddenly accelerated rotation of the prongs in the sample. During this rotation of the prongs, the applied force is decreasing.

Pictures or visual readings taken at various intervals during the application of force reveal the maximum torque applied before accelerated motion of the prongs sets in. This may be considered the torque required to break the gel structure.

This measurement is definite and reproducible, because there are no frictional forces to be overcome between the top and bottom of the torsion wire. The force delivered around the top of the wire will be the same as the force overcome at the bottom of the wire and is accurately proportional to the elastic displacement in the wire according to Hooke's law.

Torsion Wires

SELECTION AND CALIBRATION. The design of the instrument and method of calibration described below require that the torsion wires be able to suspend a small weight without change in torsional properties. The tensile properties of piano wire are sufficiently high to meet this requirement. Furthermore, if the stress applied never exceeds 50 per cent of the elastic limit, the properties of piano wire are permanent (assuming prevention of corrosion). It is very difficult to straighten piano wire which has been coiled; therefore, it is desirable to obtain straight lengths from the factory and keep them straight during preparation and use.

The principle of the torsional pendulum (*1*) was employed in calibrating the wires as follows:

A rigid brass disk, *BD*, Figure 1, of known weight and dimensions was attached to the lower end of the torsion wire, thus forming a torsion pendulum. To evaluate the period of this pendulum, the disk is rotated from its equilibrium position

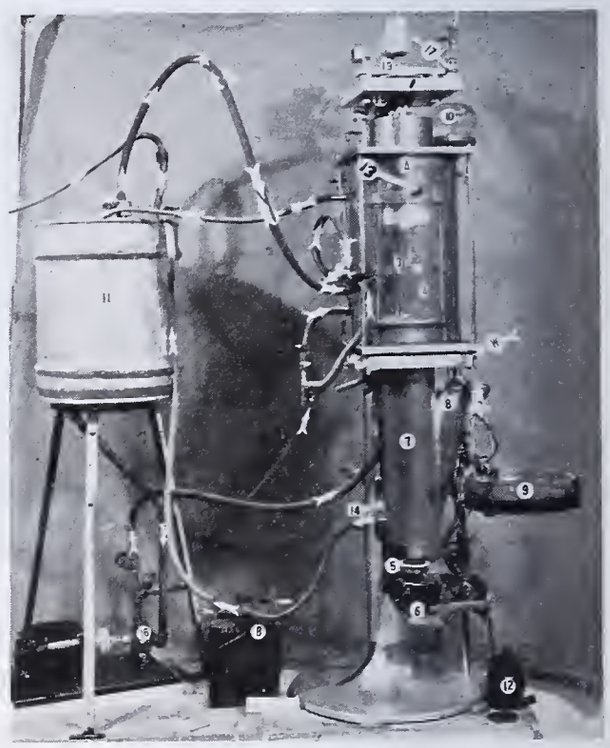

FIGURE 5. APPARATUS USED IN STUDYING BREAKING STRENGTH OF LYOGELS

1. Upper platform supporting lamp housing
2. Torsion wire
3. Pointer
4. Brush
5. Polarizer
6. Camera
7. Water jacket
8. Side tube through which camera sees stop watch, *W*
9. Dish
10. Wash bottle
11. Tank
12. Transformer for operating projection lamp bulb
13. Opening in glass water jacket so that screwdriver may be inserted to remove or replace torsion wires
14. Clamp handle
15. Slip ring and brush assembly
16. Pump
17. Crank for driving worm gear to rotate torsion wire
19. Upper ball bearing housing support
A. Glass water jacket
B. Battery for operating camera shutter
L. Lights
W. Stop watch

Arrows on tubes show directions of flow

about the vertical axis of the torsion wire. When released, the disk will begin to whirl and the number of complete oscillations during a measured interval of time can be counted. The time in seconds divided by the number of complete vibrations is the period, T, of the pendulum.

This period of the torsion pendulum is also given by the equation

$$T = 2\pi \sqrt{I/K'} \qquad (1)$$

where I is the moment of inertia of the disk and K' is the coefficient of torsional stiffness of the wire. I is the product of the mass, M, times the square of the radius, R^2. I is equal to MR^2; hence, it can be found for any given disk. Knowing T and I, the value of K' can be obtained from Equation 1. This value will be characteristic of the particular wire used in its determination.

Although all piano wires of a given diameter, length, and past history as regards tempering, stretching, etc., will have coefficients of stiffness within a certain range, it is necessary to determine the coefficient for each particular wire and tabulate it with reference to that wire only. Furthermore, a similar length of the same wire should be used to determine the elastic limit, which should be tabulated with reference to 50 per cent of its value. This value should not be exceeded in use.

If a wire is twisted about its axis by application of a torque, it has been found experimentally that the angle in radians, Θ, through which the wire is twisted is proportional to the applied torque. That is,

$$T = K'\Theta \qquad (2)$$

In which K' is again the coefficient of torsional stiffness evaluated in Equation 1 and is the characteristic of a given wire mentioned above for tabulation. The value of Θ in radians can be determined for the reading obtained by referring the upper and lower torsion wire pointers to the circular protractor scale in the apparatus. It is the difference between the two readings at any time. The value of T thus calculated by use of Equation 2 has dimensions in centimeter-dynes and is used in calculating the specific ultimate strength as discussed below.

Specific Ultimate Strength

An expression for specific ultimate strength for use with this instrument may be derived as follows:

Consider the plastic set in a circular trough having fins to prevent slippage of the sample at the walls. Consider also that the force is applied by means of a set of prongs made from a section of Duralumin or other suitable tubing, closed at the upper end by a disk to which the torsion wire is fixed and slitted in the lower end to form rounded-edged prongs dipping into the sample. Assume that there is a sufficient number of prongs to produce a circular break in the test specimen and that the surfaces are treated to prevent establishment of forces of adsorption between sample and prongs. The break will be in the form of a vertical cylindrical path or sleeve having a radius equal to that of the circle in which the prongs are set and having a height equal to the depth of the prongs in the gel. The area of this cylindrical break will be equal to the circumference of the circle diminished by the summation of the circular lengths of the prongs, then multiplied by the depth of the prongs in the specimen. The torque required to produce breakage is proportional to this area. Let

T = torque, cm.-dynes
r = radius of circle in which prongs are set, cm.
h = depth of prongs in specimen, cm.
Σl = summation of circular lengths of prongs, cm.
β = specific ultimate strength, dynes divided by sq. cm.

Maximum torque applied in breaking specimen divided by radius at which this torque is acting is equal to the maximum total applied force; and also is equal to the ultimate force which can be resisted by the given vertical cylindrical area of the specimen in which break occurs. This ultimate force is given by the cross-sectional area of breakage times specific ultimate strength of the specimen. Consider the cross-sectional area of breakage to be the summation of the circular areas of the segments removed from the tubing in making the slits mentioned above. That is,

$$T/r = [(2\pi r) - (\Sigma l)]\, h\beta \qquad (3)$$

or

$$\beta = T/r\,[(2\pi r) - (\Sigma l)]\, h = \text{specific ultimate strength of the sample in terms of dynes per square centimeter} \qquad (4)$$

The above derivation assumes no forces of adsorption between prongs and test specimen. This condition might be approached by coating the prongs with a suitable adsorbed film before the test.

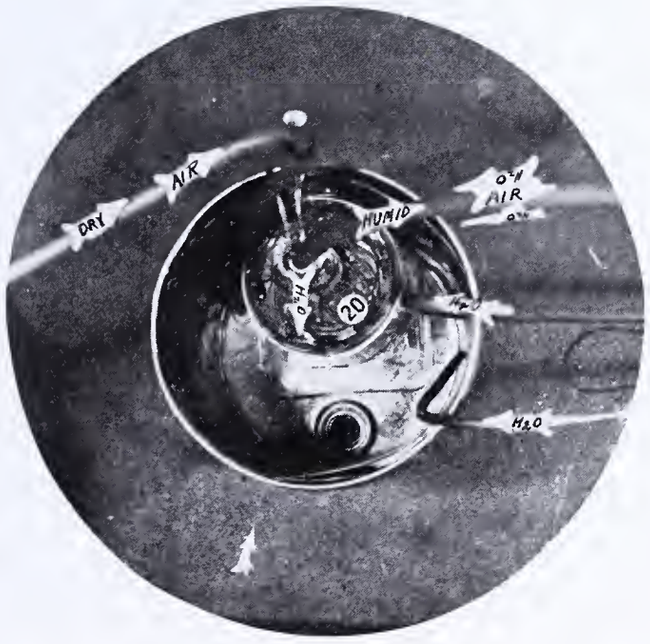

FIGURE 6. LOOKING DOWN ON TANK 11 OF FIGURE 5
20. 2-gallon bottle with bottom removed. Coiled copper tube air sparger may be seen beneath bottle

If the torque is alternately applied and released reversibly, a value of T may be found which is a maximum beyond which the prongs no longer return to their original position with respect to the protractor scale after the applied torque has been released. By substituting this value of T in Equation 4 the proportional limit may be calculated beyond which creep will occur in the sample. However, the ultimate strength is most important and is found by substituting the maximum value of T observable at the start of accelerated rotation of the prongs with simultaneous decrease in torque.

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Measurement of Flow Properties with the Gardner Mobilometer

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THE rheological properties of various plastic soft solids as reported by use of the mobilometer have never been stated in absolute units and, therefore, comparison with the results obtained with other instruments is not easy. It is the purpose of this paper to show how one mobilometer has been calibrated to give results in absolute units. A comparison of the results obtained when several materials are measured with three different types of disk is presented.

Cornthwaite and Scofield (*5*) have stated that the mobilometer is suitable for precise work in consistency studies, although they have not given any indication of the order of magnitude of the precision obtainable. This paper, therefore, also reports a study of the precision that may be obtained.

The mobilometer was first described by Gardner and Parks (*6*) for the control of the flow properties of paints, enamels, and pigmented lacquers. Its dimensions were later standardized in the Gardner laboratory by Sward and Stewart (*12*). It has been used by numerous workers to report the rheological properties of several systems of materials. Gardner and Van Heuckeroth (*7*) have applied the instrument to food products, mineral oils, petrolatum, coal tar, etc. Gray and Southwick (*8*) have used the instrument in their studies of the mayonnaise emulsion. Turnbull (*13*) and McIntyre and Irwin (*11*) have used it to study the flow properties of ceramic clay slips. Baldeschwieler and Wilcox (*1*) have utilized the instrument as a viscometer for viscous mineral oils. The writer has found use for it in a study of the rheological properties of adhesives, particularly those used in the installation of various floor coverings.

An ideal instrument for use in the study of the flow properties of industrial materials should satisfy the following requirements:

1. It should work on the right principle—i. e., it should be possible to vary the rate of shear so that the fundamental flow properties are immediately apparent.
2. It should be relatively precise and the degree of precision must be known.
3. It should be adaptable to a rather wide range of consistencies.
4. From the data collected, it should be possible to calculate the results in absolute units.
5. The instrument should be easily manipulated, so that a relatively inexperienced operator can be trusted to collect data.
6. It should be sturdy in construction.
7. It should be easily cleaned.
8. The speed at which determinations can be made should be high, so that the large amount of data usually necessary can be collected without too much expenditure of time.

In control instruments, the first four requirements are often sacrificed, and the results are of little value if the system being measured is altered more than is slightly incidental to the consistency being controlled. The results so collected are usually of no value as research data.

An industrial research instrument must meet all the above requirements, with emphasis on the first four. The Gardner mobilometer is a control instrument which can be made to satisfy all points if the degree of precision is known and if the results are calculable in absolute units. It can, therefore, be used as an industrial research instrument.

Description of the Gardner mobilometer has been adequately covered in the literature. Essentially, no fundamental changes have been made in the shape or size of the disks or cylinders of the commercially available instruments. Combes, Ford, and Schaer (*4*) have replaced the disks by a perforated cone for use in measuring the consistency of greases.

The writer has found a glass tube fitted in a stopper on the top of the cylinder helpful in dealing with materials containing volatile solvents. Continued exposure of such materials to the air currents of the room causes loss of solvent which sometimes results in deleterious skinning of the exposed surface. High loss of solvent will, of course, produce inaccuracies. The use of the tube decreases this source of error to a large degree. The tube consists of a piece of standard 20-mm. glass tubing 180 mm. long, drawn down at the upper end, and fitted into a No. 9 rubber stopper at the lower end. The clearance between the drawn end of the tube and the moving plunger rod should be about 1 mm. It is important that the drawn end be carefully fire-polished.

In a study of the mobilometer, one is impressed by a similarity between it and an instrument described by Clarvoe (*3*), which was designed for measuring the consistency of roofing putties and fibrous roof coatings, and was developed without knowledge of the existence of the mobilometer. In construction, the Clarvoe instrument varies essentially only in that the diameter of the cylinder is greater and no disk is used on the end of the plunger rod when measuring stiff putties. Materials of lower consistency are measured with a ball on the end of the rod. The perforated disks of the Gardner instrument are not suitable for use with fibrous materials, but it has been suggested that the ball tip principle be borrowed from the Clarvoe instrument, for this purpose.

Procedure

A standard procedure has been employed in all calibrations and observations. All work was performed in a constant-temperature room held at 22 ± 0.5° C., although an effort was made to keep the temperature of the material being measured more closely adjusted. This eliminated the necessity of a water bath, but decreased the accuracy of calibration and observation in varying degrees, depending on the temperature coefficient of the material.

The sample was brought to temperature and poured into the cylinder of the mobilometer to a height of within 2 cm. from the top. In the case of a viscous liquid the air bubbles were expelled by merely allowing the cylinder to stand undisturbed for a time. In the case of highly plastic materials, the entrapped air was eliminated by gently and steadily tapping the bottom of the cylinder on a resilient surface for several minutes. The cylinder was screwed into the base and the rod-guide was clamped in place. The top of the guide clamp in contact with the cylinder was kept at approximately the same height as the level of the contents of the cylinder. In adjusting the weights producing the flow (the weight of disk, rod, and weight pan plus the added weight) no attempt was made to keep the minimum weight of the system 100 grams, as is usually done. In fact, an effort was made to have available a lower minimum shearing weight in some cases, so that the instrument could be applied to more fluid materials. This was done by removing the weight shot from the hollow rod (the shot is placed in the rod by the maker in order to standardize the weight of disk, rod, and weight pan, so that this combination will weigh 100 grams). In this manner, a shearing weight of less than 50 grams was available. At a chosen shearing weight, the time for several plunges was recorded, having the plunger in a different position radially for each determination. At certain positions, the frictional forces of the instrument appear to be at a minimum. Reference is made below to means of minimizing the effects of friction due to the crudity of construction. The position of minimum time was chosen and an effort was made to keep this same radial position in subsequent calibration and

observation. The time was recorded for a fall of 10 cm., this 10 cm. being chosen so that the disk will fall through that volume of material in approximately the middle section of the cylinder, in order to minimize any end effects which might be inherent.

In the case of true liquids, after each plunge the rod was not wiped clean of adhering material, but at least 2 minutes' draining time was allowed, so that the material could flow from the rod before the next plunge was made. This practice was adopted because the use of the glass air current shield in certain cases made wiping after each plunge impractical. The amount of liquid adhering to the rod after 2 minutes' draining time was usually less than 1 gram for 10 cm. of exposed rod. In the case of plastic soft solid materials of considerable yield value, frequent wiping was necessary, as layers of the material tended to build up with successive plunges and withdrawals. This point of procedure was not particularly desirable but seemed practical. An average time for at least three falls for each shearing weight was used in the calculations.

Theoretical

The Gardner mobilometer in its operation applies the multiple orifice extrusion principle. Each hole in the disk may be considered to be a short tube of large diameter. The clearance between the disk and the cylinder wall is wholly operative in flow in the case of the blank disk and only partially operative in the cases of the disks in which there are holes. We may consider the operation of the blank disk, as applying flow between cylinders. Observation shows that the disk, however, does not travel coaxially to the cylinder, but rather travels so that the disk is closer to the cylinder at one point. The flat face surfaces of the disk will also offer resistance to flow. The rod may be considered to apply approximately the coaxial cylinder type of fall. The resistance offered by the passage of the rod is negligible, however, as will be noted if we try to time the fall of the rod on which there is no disk. Unless we are dealing with materials of very heavy consistency, the time is so small that it is not measurable by means of ordinary stop-watch technique.

It can be seen that in the mobilometer the types of flow are complicated, and the results of calculations based purely on the dimensions of the instrument would probably mean very little.

Newton's fundamental law of viscous flow states that when two parallel planes separated by distance s are sheared by a force, F, per unit area, the velocity, v, which one plane travels with respect to the other will be proportional to the coefficient of viscosity, η, of the material separating the planes.

Thus,

$$\eta = \frac{Fs}{v} \tag{1}$$

or

$$\eta = Fst \tag{2}$$

where t = time for constant amount of shear $= \frac{1}{v}$. Here s may be considered to be an instrumental constant, K, since the value of s depends only on an instrumental adjustment. K, in this case, can be evaluated either by calibration against a liquid of known viscosity or from the dimensions of the instrument. The general expression

$$\eta = KFt \tag{3}$$

or

$$\frac{1}{t} = \frac{KF}{\eta} \tag{4}$$

may be derived. Equation 3 is a general expression into which the more complicated formulas of all instruments may be transformed.

Thus in the case of the more complicated type of flow, the flow through a capillary tube, Poiseuille has given the law

$$\frac{V}{t} = \frac{\pi PR^4}{8l\eta} \tag{5}$$

where V = volume of flow, t = time of flow, P = pressure, R = radius of capillary, and l = length of capillary, which yields

$$\frac{1}{t} = \frac{KF}{\eta} \quad \text{or} \quad \eta = KFt \qquad \text{(4) or (3)}$$

where

$$F = \frac{RP}{2l} \tag{6}$$

and

$$K = \frac{\pi R^3}{4V} \tag{7}$$

All the terms of K depend on dimensions of the instrument, so here again K may be regarded as an instrumental constant. Dimensional analysis shows the value of KF to be dynes per sq. cm., in both examples. Here again, the value of K can be obtained either by calibration against a liquid of known viscosity or from the dimensions of the instrument.

In the case of the mobilometer where the type of flow is much more complicated than in parallel plate or capillary flow, the expression

$$\frac{1}{t} = \frac{KF}{\eta} \quad \text{or} \quad \eta = KFt \qquad \text{(4) or (3)}$$

also applies, although the value of K cannot be obtained from dimensions of the instrument but must be evaluated by calibration against a standard liquid. Here again, however, the dimensions of KF are dynes per sq. cm.

The mobilometer has been designed to measure the flow properties of materials which do not behave as true liquids—i. e., those materials which behave as plastic soft solids or non-Newtonian liquids. In the case of plastic solids, Bingham (*2*) has given an equation which expresses their behavior.

$$\frac{V}{t} = \frac{\pi R^4 \mu}{8l}(P - p) \tag{8}$$

where μ = mobility (sq. cm. per dyne-second) and p = yield value due to structure (dynes per sq. cm.). The values of μ and p are, respectively, the slope and intercept at the abscissa of the straight line asymptotic to the curve obtained when the rate of shear, V/t, is plotted as the ordinate against the shearing stress, P, as the abscissa. The yield value, p, has the same dimensions as P. The mobilometer gives curves such that the points measured, for the most part, fall on the section of the curve which is coincident with the asymptote. In fact, it is impossible in most cases to detect curvature graphically in this section of the curve, so it may be considered to be a straight line.

Equation 8 may be given the same treatment as Equation 5 to give

$$\frac{1}{t} = \mu(KF - Kf) \tag{9}$$

Equation 4 is specific for application in the measurement of the viscosity of a liquid. Equation 9 is for application to the mobilometer in the case of a plastic solid. The value of K for the mobilometer, obtained from calibration with a standard liquid, is an instrumental constant and it can, therefore, be used in Equation 9. The actual values of K obtained in the cases of the different instruments (parallel plates, capillary tube, or mobilometer), of course, will not be equal.

The values of μ and Kf, necessary as physical constants to express the flow characteristics of a plastic solid, can be calculated by the method of averages (*10*) using values of $1/t$ and KF obtained experimentally. Only those values which occur

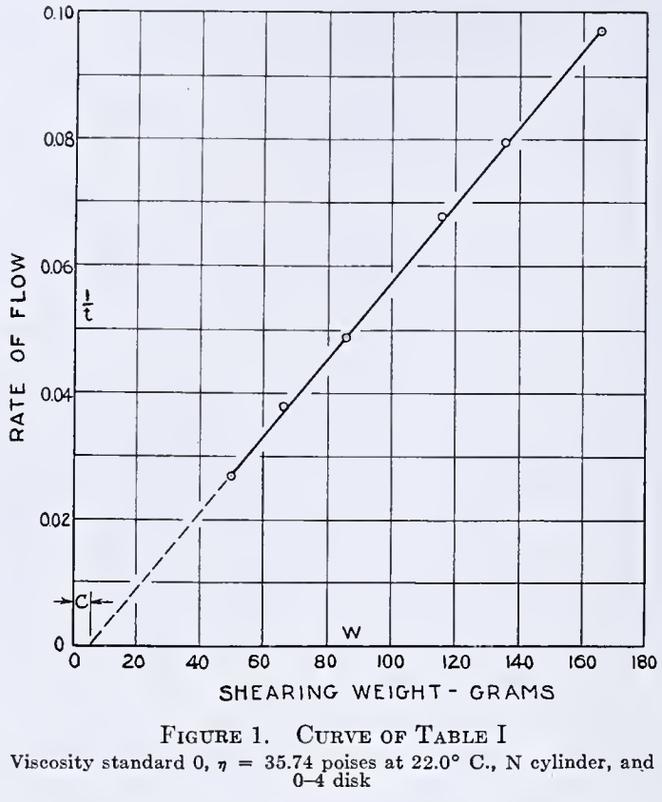

FIGURE 1. CURVE OF TABLE I
Viscosity standard 0, η = 35.74 poises at 22.0° C., N cylinder, and 0–4 disk

on the linear portion of the curve should be used in the calculations.

Calibration

A Bureau of Standards calibrated viscosity standard liquid having a viscosity of 35.74 poises at 22.0° C. was used to calibrate the instrument and thus obtain the instrumental constants for various combinations of disks and cylinders. Two sets of three disks—i. e., one blank, one 51-hole, and one 4-hole disk in each set—were calibrated. One set had been in use for some time and one set was recently purchased from the Gardner laboratory; the former set is identified by the prefix O- and the latter, by N-. Five cylinders were calibrated, four old and one recently obtained, identified by the symbols I, II, III, IV, and N. The practice followed has been outlined above under the heading of Procedure.

Corrections

In Table I are given the times necessary for a 10-cm. fall of the plunger rod fitted with a four-hole disk, using various weights to produce the shear. It was found that the straight line obtained when the rates of fall—i. e., the values of $1/t$—were plotted against the shearing weights in grams, did not go through the origin, but instead had an intercept at a small value on the abscissa. Inspection shows that the total weight producing the shear is not just overcoming the viscous resistance of the liquid, but a portion of the weight is being used to overcome the buoyant force of the submerged disk and rod. In order to have the shearing weight corrected so that it will equal the weight producing shear, the buoyant effect correction must be subtracted from the shearing weight. Equation 3 can be expressed

$$\eta = K(W - C)t \qquad (10)$$

where $F = W - C$

Figure 1 shows the curve obtained when the values given in Table I are plotted. Constants A and C of the equation representing the curve were calculated by the method of averages.

$$W = A\frac{1}{t} + C \qquad (11)$$

where W = shearing weight—i. e., the weight of disk, rod, and weight pan plus added weights; C = correction constant, the need of which is attributed mainly to the buoyant effect; and A = straight line equation constant.

It will be noted that

$$K = \frac{\eta}{A} \qquad (12)$$

The value of C can be approximated by calculation from the dimensions of the submerged parts and the specific gravity of the material under measurement.

The above statement has not taken into consideration any frictional forces due to contact of the moving disk and the inside of the cylinder, which are in opposition to the weight and are therefore in the same direction as the buoyant force. These frictional forces cannot be calculated from dimensions of the instrument and may be disregarded in the cases of well-machined disks and cylinders where the friction, if effective at all, is very small. The frictional forces arise from poorly machined working parts; those disks and cylinder walls which appeared to be rough had higher values of C than the smoother appearing ones. If accurate results are desired, the moving parts should be accurately machined and carefully finished. It was noted that those disks which had been used for some time had lower values of C than the newer ones. If careful technique is practiced, it is logical to conclude that any gross discrepancies in measurement or low precision may be accredited to large values of frictional forces.

TABLE I. CALIBRATION WITH BUREAU OF STANDARDS VISCOSITY STANDARD

(η = 35.74 poises at 22° C.; t = time of fall of 10 cm. with weight W, using a 4-hole disk)

A = 1.645, C = 5.4, K = 0.02173

W Gram	t Sec.	$1/t$	$F = W - C$	η Calcd.	Error %
49.3	37.3	0.02681	43.9	35.58	0.4
65.9	27.2	0.03676	60.5	35.76	0.1
85.9	20.5	0.04878	80.5	35.86	0.3
115.9	14.8	0.06757	110.5	35.54	0.6
135.9	12.6	0.07937	130.5	35.73	0.0
165.9	10.3	0.09709	160.5	35.92	0.5
			Av.	35.73	0.3

TABLE II. VALUES OF INSTRUMENTAL CONSTANTS

Cylinder No.	Disk No.	A	C	$K = \frac{\eta}{A}$	$k = \frac{V\eta}{A}$	Average Deviation %
N	N-B	220,600	16.6	0.0001620	0.01925	1.9
N	N-51	16,120	16.7	0.002217	0.2634	1.0
N	N-4	1,931	6.4	0.01851	2.200	1.5
N	O-B	57,820	−0.2	0.0006181	0.07345	0.4
N	O-51	14,600	2.1	0.002448	0.2909	0.3
N	O-4	1,645	5.4	0.02173	2.583	0.3
I	O-4	1,702	2.9	0.02100	2.495	0.5
II	O-4	1,705	3.3	0.02096	2.491	0.9
III	O-4	1,639	4.7	0.02180	2.591	0.4
IV	O-4	1,691	3.0	0.02113	2.511	0.1
Average	O-4 Disk	1,676	3.9	0.02132	2.534	0.4

The error introduced by not wiping the rod clean of adhering liquid between determinations is not large if sufficient time is allowed for drainage. In the case of the calibrating liquid less than 1 gram of material adhered after 2 minutes' draining time. As plastic soft solids do not drain properly and consequently add considerably more weight, they should be wiped after each determination if high precision is desired. The percentage error introduced by not removing the adhering film, however, in the case of these plastic materials, may not be very large, since the weights necessary to produce flow in these materials are usually rather high.

There is a different value of the instrumental constant for each combination of disk and cylinder. Table II gives the values of the instrumental constants, K, along with the value

f A from Equation 11 which is used in calculating K and k. n order to calculate k it is necessary to know the value of V, ne volume of flow. This value, calculated from the dimen- ons of the instrument, is 118.8 cc. for 10-cm. fall.

The constants are given for the combination of one cylinder nd different disks and for one disk and several cylinders. 'he wide variation in the values of K, for the disks which are ıpposed to be interchangeable, makes the necessity of alibration of the various disks immediately apparent, if esults of measurement of flow properties with one mobil- meter are to be compared with those of another. The values f K obtained when the different cylinders are used inter- hangeably do not vary so widely as do the values of K using ne "interchangeable" disks.

The average value of the buoyant force correction, C, for isk O-4 in combination with the various cylinders is 3.9 rams. This value compares very well with the value calcu-

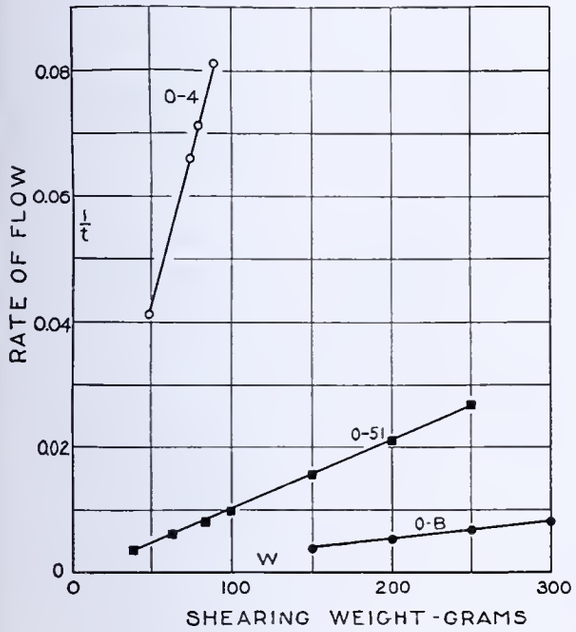

Figure 2. Manila Copal Resin Solution in Alcohol
58.4% by weight

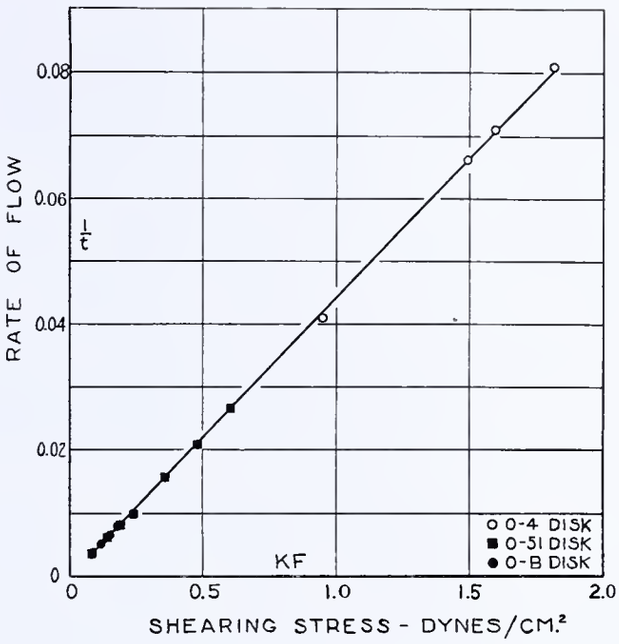

Figure 3. Manila Copal Resin Solution in Alcohol
58.4% by weight, plotted in absolute system units

lated from the dimensions of the submerged parts, the mean volume of the submerged parts being 5.7 cc. This would give 4.9 grams' buoyant force, since the specific gravity of the calibrating liquid is 0.86. The 1.0-gram difference between average C and buoyant force calculated from the dimensions is attributed to the weight of calibrating liquid adhering to the rod.

The frictional force is negligible in the case of disk O-4, as it is with the other older, smoother-surfaced disks O-B and O-51. Higher frictional force is, of course, associated with rougher working surfaces. Also, as summarized in Table II, the average percentage deviations between the known viscosity of the calibration liquid and the values of viscosity, calculated using Equation 3, are higher for the new disks than for the smooth ones. It will be noted that the value of C for disk O-B is -0.2. This out-of-line value is attributable to calibration inaccuracy, but since the shearing weights used in this case were in the range of 350 to 1100 grams, the resulting percentage deviation between -0.2 and 3.9 values of C was not excessive. The percentage deviation in this case was of the same order as for the other O-set disks.

Determinations

The viscosity of a clean, concentrated solution of Manila copal resin in denatured alcohol (Special Denatured Formula No. 1) was measured. The solution was prepared by dissolving the CNE grade of this natural resin in the alcohol, allowing the dirt and other insoluble material to settle, and using the clear supernatant liquid. Analysis of this solution showed its concentration to be 58.4 per cent by weight. Table III presents the data obtained when the viscosity was measured using O-4, O-51, and O-B disks and N cylinder. In Figure 2, the reciprocal of the time required for 10-cm. fall of the plunger is plotted against the weight producing the fall. Each numerical value of t is an average of the times of at least three falls at the same weight. In order to calculate the viscosity from the data, it is necessary to subtract from the shearing weight a correction for the buoyant force. For purposes of calculation, the average submerged volume will be taken as 6 cc. To calculate the shearing force—i. e., the weight overcoming viscous resistance—from the shearing weight, Equation 13 is used.

ABLE III. Gardner Mobilometer Data for 58.4 Per Cent Alcoholic Solution of Manila Copal Resin

)-4, O-51, and O-B disks with N cylinder. Temperature, 22° C. C, 5.7)

W	t	F	KF	$1/t$	$\eta = KFt$	Deviation from Mean Value of η
Grams	Sec.		Dynes/ sq. cm.			%
			Disk O-4, K = 0.02173			
49.3	24.3	43.6	0.947	0.041	23.0	0.0
74.2	15.2	68.5	1.489	0.066	22.6	1.8
79.2	14.1	73.5	1.597	0.071	22.5	2.2
89.2	12.4	83.5	1.814	0.081	22.5	2.2
						Av. 1.5
			Disk O-51, K = 0.00244S			
39.1	286.7	33.4	0.0818	0.00349	23.4	1.7
64.0	164.1	58.3	0.143	0.00609	23.4	1.7
84.0	123.2	78.3	0.192	0.00812	23.6	2.5
100.0	101.2	98.3	0.241	0.0099	24.3	5.3
150.0	64.6	148.3	0.363	0.0155	23.4	1.7
200.0	47.9	198.3	0.485	0.0209	23.2	0.9
250.0	37.5	248.3	0.608	0.0267	22.8	0.9
						Av. 2.1
			Disk O-B, K = 0.0006181			
150.2	262.8	144.5	0.0893	0.00380	23.5	2.1
200.2	188.1	194.5	0.1202	0.00531	22.6	1.8
250.2	148.3	244.5	0.1511	0.00674	22.4	2.7
300.2	122.2	294.5	0.1820	0.00818	22.2	3.6
						Av. 2.5
				Mean value	23.0 poises	2.0

$$F = W - 6\rho \qquad (13)$$

where ρ = specific gravity of the material being measured. In the case of this solution where the specific gravity equals 0.95, the buoyant force correction is equal to 5.7. After calculating the force, F, necessary to shear the liquid, the calibration constant, K, may be used to calculate the absolute viscosity by use of Equation 3.

From the shearing force, F, and the calibration constant K, the shearing stress in dynes per square centimeter can be obtained. In Figure 3 the rate of 10-cm. fall (or rate of flow of the liquid) is plotted against this shearing stress, KF. The data represented in Figure 2 by three curves can be now represented by one curve. In Table III are also presented the calculated values of the viscosity of this solution at various rates of flow, along with the per cent deviation from the mean calculated value of viscosity. The average deviation is 2.0 per cent. It will be noted that those values of viscosity corresponding to low rates of flow tend to have a greater deviation than the higher rate values; that those viscosities obtained using the O-B disk have higher deviation than those obtained with O-51 and O-4; and similarly O-51 has higher values than O-4. The explanation of this is that probably the incalculable frictional forces are present and are exerting a greater influence at the lower rates of fall.

If to this Manila copal resin solution is added some considerable quantity of a mineral filler, the flow properties will change from those of a viscous liquid to those of a plastic soft solid and the rate of shear will no longer be proportional to the shearing stress.

To 100 parts by weight of this 58.4 per cent resin solution 80 parts of kaolin were added. This resulted in a mixture which was 21.8 per cent by volume of filler. Table IV contains the data collected using the three types of disk. The specific gravity of this mixture is 1.33, making the calculated buoyant force correction, C, equal to 8.0. Figure 4 shows curves resulting when the rate of 10-cm. fall is plotted against the shearing weight. If by using the calibration constants we calculate the shearing stress and plot this against the rate of fall, we obtain the curves shown in Figure 5.

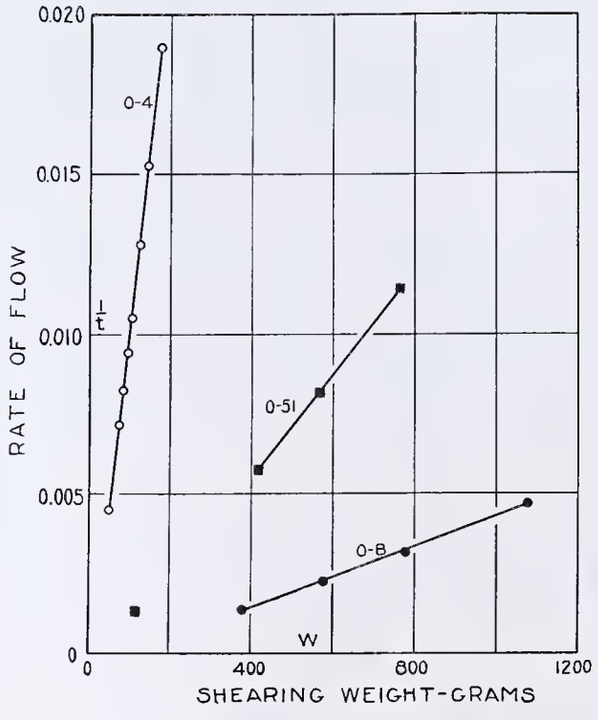

FIGURE 4. MIXTURE OF KAOLIN AND MANILA COPAL RESIN SOLUTION

21.8% kaolin by volume

TABLE IV. GARDNER MOBILOMETER DATA FOR KAOLIN–MANILA COPAL RESIN SOLUTION

(21.8% kaolin by vol., O-4, O-51, and O-B disks with N cylinder. C, 8.0

W	t	F	KF	$1/t$	KF (Calcd.)	Deviation
Grams	*Sec.*		*Dynes/ sq. cm.*			%
			Disk O-4, K = 0.02173			
49.3	222.2	41.3	(0.897)	0.00450	(0.967)	(8.1)
74.2	139.9	66.2	1.438	0.00715	1.457	1.3
84.2	121.5	76.2	1.656	0.00823	1.656	0.0
94.2	106.5	86.2	1.873	0.00939	1.870	0.2
104.2	95.4	96.2	2.090	0.0105	2.074	0.8
124.2	77.9	116.2	2.525	0.0128	2.498	1.0
144.2	65.4	136.2	2.960	0.0153	2.959	0.1
174.2	52.7	166.2	3.612	0.0190	3.640	0.8
					Av.	0.7
			Disk O-51, K = 0.002448			
114.0	775.4	106.0	(0.259)	0.00129	(0:327)	(26.3)
414.0	174.2	406.0	0.994	0.00574	0.998	0.4
564.0	123.1	556.0	1.361	0.00812	1.357	0.3
764.0	87.7	756.0	1.851	0.01140	1.851	0.0
					Av.	0.2
			Disk O-B, K = 0.0006181			
376.2	734.5	368.2	0.228	0.00136	0.231	1.3
576.2	448.8	568.2	0.351	0.00223	0.347	1.1
776.2	319.0	768.2	0.475	0.00313	0.467	1.7
1076.2	216.1	1068.2	0.660	0.00463	0.667	1.1
					Av.	1.3

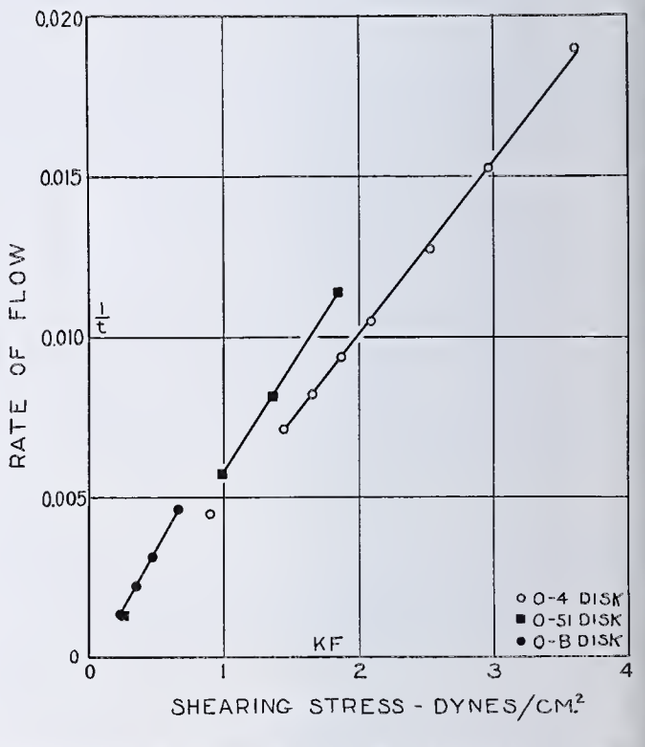

FIGURE 5. MIXTURE OF KAOLIN AND MANILA COPAL RESIN SOLUTION

21.8% kaolin by volume, plotted in absolute system units

The curves for the three disks, while apparently straight lines, do not coincide as with the resin solution without filler. The explanation of this phenomenon is that the rate of fall of the plunger rod (or rate of flow of the material) and the rate of increase of shear of the material are the same only in the case of true liquids. In the plastic soft solids, "plug flow" is exhibited. Plug flow is an extrusion mechanism with a high shear rate in the region near the wall of a tube, but little or no shear in the central section of the material being forced through the tube. The structure of the material near the wall is being violently disturbed while the center or "plug" suffers little change. The layer in a high rate of shear acts as a lubricant for the central plug. The larger the diameter of

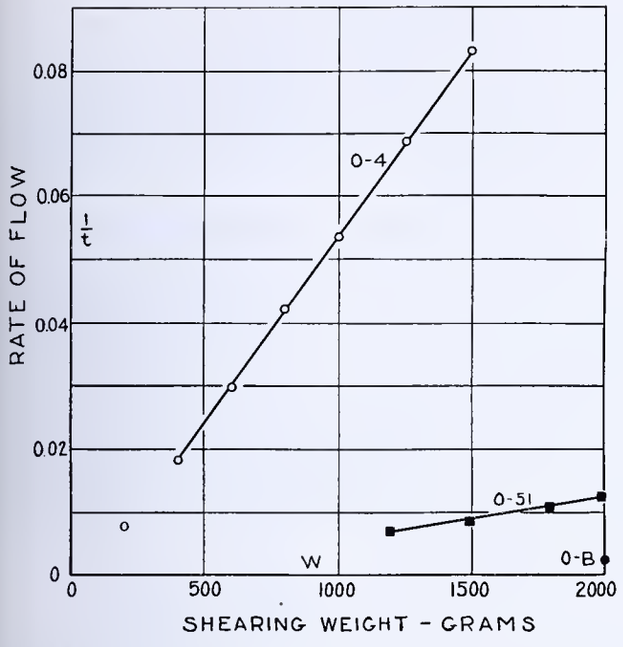

FIGURE 6. MIXTURE OF KAOLIN AND MANILA COPAL RESIN SOLUTION
26.8% kaolin by volume

e tube through which the plastic material flowing, the less the cross-sectional area the layers of breakdown on application a given shearing stress, and, therefore, e greater the tendency for this plug w effect. Hall (*9*) has experienced this enomenon of flow rates, higher than should the case, from calculations using the ngham equation, in capillaries in his measements of the plasticity of clays. Obously this phenomenon will be a source of sagreement between results obtained with rious types of disks of the mobilometer, t the discrepancy is attributable to the uctural properties of the material and k of complete applicability of the Bingham uation, and not necessarily to any cortable instrumental deficiencies.

TABLE VI. GARDNER MOBILOMETER DATA FOR KAOLIN–MANILA COPAL RESIN SOLUTION

(31.2% kaolin by volume, O-4 disk with N cylinder. *C*, 9.0. *K*, 0.02173)

W	*t*	*F*	*KF*	1/*t*	*KF* (Calcd.)	Deviation
Grams	*Sec.*		*Dynes/ sq. cm.*			%
400.0	210.8	391.0	8.50	0.0047	8.66	1.8
600.0	122.1	591.0	12.87	0.0082	12.77	0.8
800.0	84.4	791.0	17.19	0.0118	17.13	0.3
1000.0	64.8	991.0	21.53	0.0154	21.39	0.6
2000.0	29.5	1991.0	43.26	0.0339	43.39	0.3
					Av.	0.8

Equation 14 can be used to calculate the constants of the straight lines shown in Figure 5.

$$KF = R\left(\frac{1}{t}\right) + Kf \qquad (14)$$

The low shear rate values, which are represented by points that do not fall on the straight line, were not used in the calculation of the constants of the straight line. From the calculated constants R and Kf, the calculated values of KF can be obtained. These data are given in Table IV, with the values of deviation between observed and calculated values of KF. The average value of the deviation is 0.7 per cent,

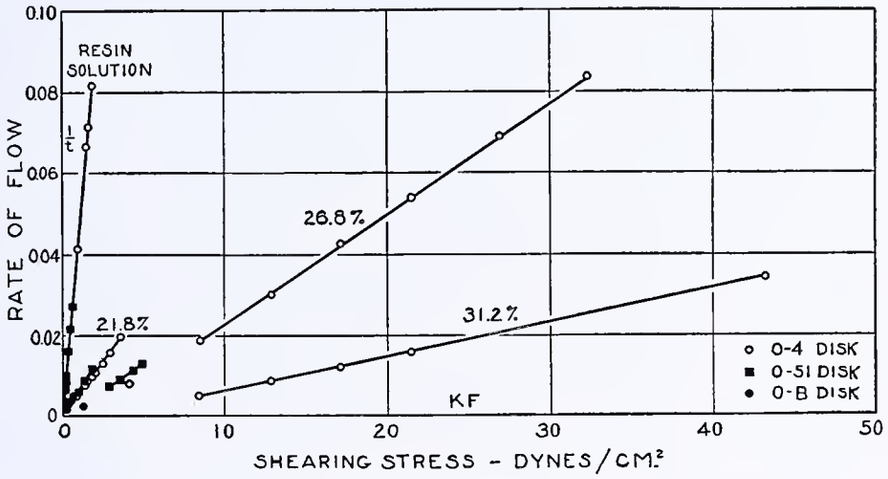

FIGURE 7. CALIBRATED GARDNER MOBILOMETER RESULTS
Manila copal resin solution and kaolin resin solution mixtures, plotted in absolute system units

BLE V. GARDNER MOBILOMETER DATA FOR KAOLIN–MANILA COPAL RESIN SOLUTION

(26.8% kaolin by volume, O-4, O-51, and O-B disks with N cylinder *C*, 8.5)

W	*t*	*F*	*KF*	1/*t*	*KF* (Calcd.)	Deviation
Grams	*Sec.*		*Dynes/ sq. cm.*			%
			Disk O-4, K = 0.02173			
200.0	129.4	191.5	(4.16)	0.0077	(4.58)	(10.1)
400.0	54.5	391.5	8.51	0.0183	8.50	0.1
600.0	33.4	591.5	12.85	0.0299	12.78	0.5
800.0	23.7	791.5	17.20	0.0421	17.28	0.5
1000.0	18.7	991.5	21.55	0.0535	21.49	0.3
1250.0	14.6	1241.5	26.98	0.0686	27.06	0.3
1500.0	12.0	1491.5	32.41	0.0830	32.38	0.1
					Av.	0.3
			Disk O-51, K = 0.002448			
1189.8	143.7	1181.3	2.89	0.0069	3.00	3.8
1489.8	118.1	1481.3	3.63	0.0084	3.52	3.0
1789.8	93.7	1781.3	4.36	0.0107	4.31	1.1
1989.8	80.6	1981.3	4.85	0.0124	4.90	1.0
					Av.	2.2
			Disk O-B, K = 0.0006181			
2000.2	435.0	1993.5	1.232	0.0023	...	..

not, of course, including in the deviation the nonlinear, low-shear rate, calculated value of KF, enclosed in parenthesis in Table IV.

Table V gives the data obtained when a mixture of 100 parts of the 58.4 per cent resin solution and 105 parts of kaolin, to give a 26.8 per cent by volume mixture (specific gravity = 1.42), is measured with the three disks. This material could be handled with the O-4 and O-51 disks; with the O-B disk the material was apparently too heavy in consistency to handle practically. The curves for rate of fall *vs.* shearing weight and rate of fall *vs.* shearing stress are given, respectively, in Figures 6 and 7. The calculated values of KF and the per cent deviation of these calculated values from the observed values are given in Table V, the average being 1.1 per cent. Here again, the low shear rate results obtained with the O-51 disk have a greater average deviation than those obtained with the O-4 disk; also, as shown in Figure 7, the curve obtained with the O-51 disk does not coincide with that obtained with the O-4 disk.

Table VI contains the data for a mixture of 100 parts of the 58.4 per cent resin solution and 130 parts of kaolin, 31.2 per cent by volume (specific gravity = 1.50), measured with the

Table VII. Gardner Mobilometer Data for 76.8 Per Cent Alcoholic Solution of Manila Copal Resin

(O-4 disk with N cylinder. C, 6.0. K, 0.02173)

W	t	F	KF	$1/t$	$\eta = KFt$	Deviation from Mean Value of η
Grams	*Sec.*		*Dynes/ sq. cm.*			%
800.0	149.1	794.0	17.28	0.00670	2573	0.8
1100.0	110.2	1094.0	23.77	0.00908	2619	2.5
2128.1	53.5	2122.1	46.11	0.01869	2467	3.5
				Mean value =	2553 poises	2.3

mobilometer. Here it was practical to use only the O-4 disk, the material being too heavy to be accommodated by the O-51 and O-B disks. Figure 7 also shows the curve of this material. The average deviation for this material is 0.8 per cent.

A more viscous solution of Manila copal resin was prepared by allowing some of the alcohol of the 58.4 per cent solution to evaporate. Analysis of the resulting solution showed its concentration to be 76.8 per cent solids. Table VII gives the mobilometer data and the calculated viscosity, the average value being 2553 poises, with an average deviation of 2.3 per cent.

In order to ascertain whether the instrumental constant is applicable to the higher viscosities in calculating viscosity, with the same precision as in the case of viscosities of the order of magnitude of the calibrating liquid, a solution of hydrogenated rosin (28.5 per cent by weight) in methyl abietate was measured in the calibrated mobilometer. The viscosity was determined to be 840 poises at 22° C. At the same temperature, the viscosity of this solution was found to be 830 poises by a falling ball method.

Table VIII summarizes the constants of the straight lines represented by Equation 14, which were used in calculating the value of KF. The values of μ and Kf obtained with the three disks do not show good agreement in the case of the plastic soft solids, for reasons given above. The average of the values obtained with various disks can, however, be considered valuable for their order of magnitude indications.

Table VIII. Summary of Values of Constants

Composition of Material	Disk	R of Equation 14	Mobility, μ	Bingham Yield Value, Kf	Deviation through Use of Constants
				Dynes/ sq. cm.	%
58.4% alcoholic solution of manila copal resin	O-4	22.6	0.04415	..	1.5
	O-51	23.4	0.04266	..	2.1
	O-B	22.7	0.04411	..	2.5
	Mean value	23.0[a]	0.04348	0.00	2.0
Above solution plus kaolin (21.8% by volume)	O-4	184.2	0.00543	0.14	0.7
	O-51	150.7	0.00664	0.13	0.2
	O-B	133.3	0.00750	0.05	1.3
	Av.	156.1	0.00641	0.09	0.8
Solution plus kaolin (26.8% by volume)	O-4	369.2	0.00271	1.74	0.3
	O-51	344.9	0.00290	0.62	2.2
	Av.	357.0	0.00280	1.18	1.1
Solution plus kaolin (31.2% by volume)	O-4	1191.0	0.00084	3.01	0.8
76.8% alcoholic solution of manila copal resin	O-4	2553.0[a]	0.00039[a]	0.00	2.3

[a] In case of true liquid where $Kf = 0$, $R = \tilde{\eta}$, and mobility (μ) is expressed as the fluidity, ϕ.

Range of Application of Mobilometer

It is helpful to know the range of viscosity measurement possible to obtain with any instrument. Table IX is based on 50 grams as the minimum shearing weight with 2000 grams as the maximum weight. Care should be exercised when using more than 1000 grams, because greater weight may cause the rod to bend unless the weight is properly centered on the pan. In the calculations, 10 seconds is assumed to be the minimum length of time and 10 minutes the maximum practical, allowable time. Turbulence and inaccuracy of timing may be introduced at lower times.

Summary and Conclusions

The Gardner mobilometer is a control instrument which can be used in industrial research, since in its operation it employs the varying rate of shear principle, it is precise enough for industrial work, it is adaptable to a wide range of consistencies, it is easily manipulated, sturdy in construction, and easily cleaned, and the necessary data can be collected with an economy of time.

The range of application in determining the viscosity of true liquids with the mobilometer is 10^{-1} to 10^{4} poises.

Precision of the order of magnitude of 2 per cent can be obtained, if proper precautions are exercised and certain necessary corrections are employed.

The most urgent precaution to be exercised in use of the mobilometer is to be sure that the working parts are well machined. The surfaces of the disk and of the inside of the cylinder should be smooth and well polished, so as to minimize any effects of the friction set up when the surfaces of these parts come in contact.

In order to obtain the true value of the force overcoming the viscous or plastic resistance of the material, a buoyant force correction must be subtracted from the weight producing the shear. This correction is especially important where the shearing weight is relatively small.

The instrument can be calibrated with a standard liquid, so that the results can be given in absolute units by the equations:

$$\eta = K(W - 6\rho)t, \text{ for a true liquid}$$

$$\frac{1}{t} = \mu(KF - Kf), \text{ for a plastic soft solid}$$

Acknowledgment

The author wishes to thank his co-workers of the Central Technical Laboratory for helpful suggestions and valuable criticism.

Table IX. Range of Application of Gardner Mobilometer Using Three Standard Disks

(Limits of time of 10-cm., fall of plunger rod = 10 seconds to 10 minutes, limits of shearing weight = 50 to 2000 grams. Application is to true liquid.)

Disk	K (Approx.)	t = 10 Seconds: W, 50 grams	W, 100 grams	W, 2000 grams	t = 60 Seconds: W, 50 grams	W, 100 grams	W, 2000 grams	t = 180 Seconds: W, 50 grams	W, 100 grams	W, 2000 grams	t = 600 Seconds: W, 50 grams	W, 100 grams	W, 2000 grams
		Poises			*Poises*			*Poises*			*Poises*		
4	0.02	10	20	400	60	120	2400	180	360	7200	600	1200	24,000
51	0.002	1	2	40	6	12	240	18	36	720	60	120	2,400
B	0.0002	0.1	0.2	4	0.6	1.2	24	1.8	3.6	72	6	12	240

4. Disk range = 10 to 24,000
51. Disk range = 1 to 2400
B. Disk range = 0.1 to 240

or

10^{1} to 10^{4} poises
10^{0} to 10^{3} poises
10^{-1} to 10^{2} poises

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Determination of Blood Magnesium

Quantitative Spectrochemical Method

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A spectrochemical method for the determination of magnesium in blood which is accurate, rapid, and simple to perform and requires only 1 ml. of sample is described. The blood samples are first diluted with a potassium alum solution which acts both as a spectroscopic buffer and internal standard and then atomized into a spark between graphite electrodes. Analysis is made by measurement of relative intensities. The average error obtained by this method is less than 3 per cent.

IN GENERAL clinical practice biological fluids are only occasionally analyzed for magnesium. However, during ecent years it has become desirable to be able to determine ccurately the magnesium content of blood as well as of other iological fluids in order to ascertain its possible relationship o certain pathological conditions. A comprehensive review f the work reported in this field, including the most reliable alues for blood magnesium, is given by Myers and Muntyler (*10*). As stated in the original articles (*5*, *6*, *14*, *15*), he magnesium determinations were made by chemical methds of analysis. However, because of the limited amount of ample available, it is necessary to have a method requiring nly a few milliliters for the analysis. For this reason the ossibilities of a spectrochemical method embodying speed, ccuracy, and minimum sample requirement have been inestigated.

A number of methods for the spectrochemical analysis of iological fluids for various elements have been reported in he literature. Among these are the methods described by Iess, Owens, and Reinhardt (*7*) and by Thomson and Lee *12*), both of which make use of the well-known internal tandard method of Gerlach (*4*), intensity calibration of each late, and photometric measurement of the relative intensies of spectral lines as described by Duffendack, Wolfe, and mith (*2*). Both methods require wet-ashing of the sample, a me-consuming step which the method here described sucssfully eliminates.

The method used is that of atomizing a solution into a spark between graphite electrodes, the atomizer being similar to the one employed by Lundegårdh (*9*) in his flame method. Several other methods have been described for introducing solutions into a spark: the Hitchen (*13*) sparking tube for solutions, the source used by Thomson and Lee (*12*) in which the spark passes between two horizontal quartz jets from which the solution drips, and the more simple method employed by Keirs and Englis (*8*) in which the spark is formed between carbon electrodes, the upper one being hollow to permit inserting a glass capillary from which the solution drips. While each of these methods has a number of good points, the manipulations required to clean and refill the apparatus are more involved than is desirable for a method which is to be used for the routine analysis of a large number of samples.

The preliminary work on this method has been reported by Cassen (*1*). However, a number of changes and refinements in technique have been introduced in order to obtain the necessary accuracy. The three features which combine to make the method accurate, rapid, and simple to perform are use of a spectroscopic buffer which also acts as an internal standard, use of electrodes of special design, and photometric measurement of relative intensities of spectral lines.

The blood is diluted with a solution of potassium aluminum sulfate which serves both as a spectroscopic buffer and as an internal standard. It has been established by experiment that when aluminum is present in a concentration corresponding to 47 grams of $Al_2(SO_4)_3.K_2SO_4.24H_2O$ per liter of solution (using the conditions of exposure standardized upon and described under procedure for analysis) the densities of the aluminum line at 2816 Å. and the magnesium line at 2795 Å. are equal for a solution containing 0.35 mg. of magnesium per 100 ml. and also fall near the center of the straight-line portion of the characteristic curve of the photographic emulsion. Both conditions are ideal for obtaining reproducible results, and are especially desirable since this figure corresponds to 3.50 mg. of magnesium per 100 ml. of blood, which is about the average value reported for normal whole blood, the range being 2.75 to 5.00 mg. of magnesium per 100 ml. (*6*). The compound containing this internal standard, in the concentration used, is also a very effective spectroscopic buffer. The advantages of the use of spectroscopic buffers in analyzing biological materials are fully discussed elsewhere (*3*, *7*). Briefly, in this case, the problem is reduced to the determination of small amounts of magnesium in a potassium aluminum sulfate base solution; and any effects which might be introduced by the comparatively small amount of organic material present in blood or other biological fluids have been

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found by experiment to be negligible. Proof of this statement is shown by the experimental results given in Table I. Thus it is possible to analyze the samples directly after proper dilution with the internal standard stock solution. This results in a marked saving of time as compared with the time required for wet-ashing of samples and also makes it possible to adapt the method easily to the analysis of other biological fluids.

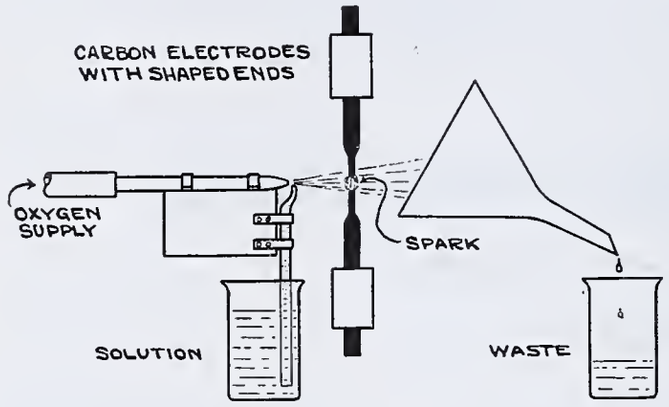

FIGURE 1. APPARATUS

The electrodes used are shaped as shown in Figure 1. These were found greatly to increase the uniformity of exposure since, as the electrodes burn away, the cross-sectional area (6.25 × 2.5 mm., 0.25 × 0.10 inch) remains constant, making it possible to use the same pair of electrodes for a large number of samples. This narrow rectangular electrode also prevents wandering of the spark, for the source of illumination is at all times in optical alignment with the slit of the spectrograph. Further, it has been established experimentally that by sparking the electrodes in a spray of distilled water for 60 seconds after each sample all traces of aluminum and magnesium are removed. This fact, together with the shape of the electrode, makes it possible to use one pair of electrodes for all the exposures on one plate. This greatly reduces the number of manipulations required and is a great saving in time.

The method of measuring relative intensities of spectral lines (*2*) has been used in order to obtain more reproducible results and to eliminate the necessity of repeating standard solutions on each plate. While it is necessary to place a calibration pattern on each plate, which is accomplished by means of a step sector, yet it is possible to run sixteen separate or eight samples in duplicate on a single plate.

Apparatus

For this work a Zeiss quartz spectrograph (chemist's model) was used. A rotating step sector (ratio 1.5) was placed directly in front of the slit for obtaining the plate calibration patterns. The atomizing unit (Figure 1) was placed 1 meter from the slit with no intervening lens system. A Hilger nonrecording microphotometer was used for making the photometric measurements. The spark source used was a condensed spark with synchronous interrupter.

Method of Analysis

PREPARATION OF SOLUTIONS. Standard solutions containing 47 grams of $Al_2(SO_4)_3.K_2SO_4.24H_2O$ per liter and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mg. of magnesium as magnesium sulfate heptahydrate per liter are prepared. An internal standard stock solution for dilution of blood samples containing 52.22 grams of potassium aluminum sulfate per liter is prepared.

DETERMINATION OF ANALYTICAL CURVE. A number of plates were made from the standard solutions using the technique described below. The blackenings of the aluminum line at 2816 Å. and the magnesium line at 2795 Å. were measured photometrically. The logarithms of the ratios of the intensities of these two lines, obtained by application of the measured blackenings to the charateristic curve drawn for each plate, were plotted against the logarithms of the corresponding concentrations of magnesium. Figure 2 gives the resulting analytical curve for the determination of magnesium in a potassium aluminum sulfate base solution.

PROCEDURE FOR ANALYSIS. The atomizer is adjusted to a rate of 15 ml. per 120 seconds. Before the first exposure and after each succeeding exposure the electrodes are cleaned by a preliminary sparking for 1 minute in a spray of distilled water. The distance between the electrodes is set at 2.35 mm. (0.094 inch) by a spacer. The blood sample is diluted by pipetting 1 ml. of blood into 9 ml. of the internal standard stock solution, and then carefully mixed until a stable uniform suspension results. The diluted blood sample or a standard magnesium solution, as the case may be, is placed in the atomizer, the stop watch being started at once; after the solution has atomized for a period of 30 seconds, the spark is started and a 30-second exposure made. The graphite electrodes are then cleaned by sparking in distilled water for 60 seconds and the procedure is repeated for a series of samples. For producing a calibration pattern on each plate (Eastman process) the step sector is rotated in front of the slit and a solution of ferrous sulfate is sprayed into the spark in order to produce lines of suitable blackening for determining the characteristic curve of the plate. The plates are rapidly processed by the procedure described by Sawyer (*11*) except that Eastman developer D-19 is substituted for D-8. The blackening of the spectral lines is then measured and the ratios of the relative log intensities of the selected pair of aluminum and magnesium lines are determined. By referring to the analytical curve the amount of magnesium present is quickly obtained.

Results and Discussion

In order to determine the accuracy of the method a number of magnesium determinations were made on whole blood, followed by redeterminations after known amounts of magnesium were added. Two different samples of blood were run in triplicate. Sample 1 was found to contain 3.10 mg. of magnesium per 100 ml. and sample 2, 3.51 mg. of magnesium per 100 ml. of whole blood. To each sample 1 mg. of magnesium as magnesium sulfate heptahydrate was added per 100 ml. of blood. These additions were made on three separate portions of each sample, and the total magnesiums redetermined spectrographically. In a similar manner 2 mg. of magnesium were added per 100 ml. to three separate portions of each sample and again the total magnesium contents redetermined. The results of these determinations are given in Table I. It is noted that the maximum error in these twelve determinations is 2.44 per cent, with an average error of 1.5 per cent.

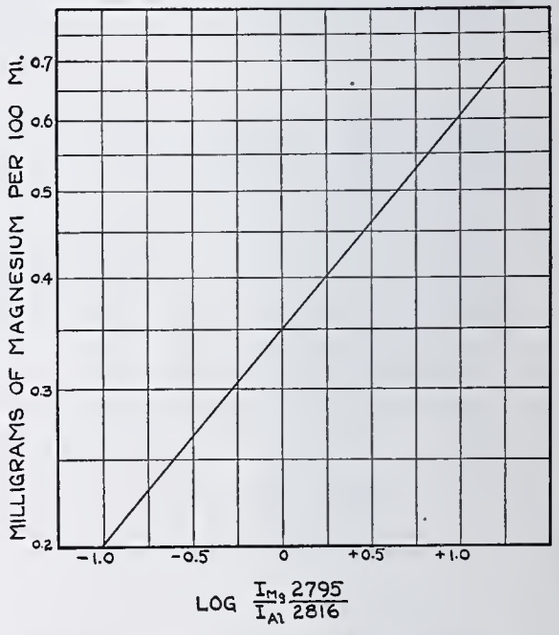

FIGURE 2. ANALYTICAL CURVE FOR DETERMINATION OF MAGNESIUM

Table I. Test Data for Accuracy of Analysis of Blood for Magnesium

Found per 100 Ml. of Original Blood	Found per 100 Ml. of Original Blood + 1 Mg. of Mg per 100 Ml.	Error[a]	Found per 100 Ml. of Original Blood + 2 Mg. of Mg per 100 Ml.	Error[a]
Mg.	*Mg.*	%	*Mg.*	%
3.10[b]	4.00	2.44	5.00	1.96
3.10	4.10	0.00	5.05	0.98
3.10	4.20	2.44	5.20	1.96
3.51[c]	4.48	0.66	5.60	1.63
3.51	4.40	2.44	5.50	0.18
3.51	4.60	1.99	5.60	1.63

[a] Per cent error based upon total amount of magnesium present in mg. per 100 ml.
[b] 3.10 is average value of three determinations of original blood sample 1.
[c] 3.51 is average value of three determinations of original blood sample 2.

Table II. Magnesium Determination in Normal Blood

Sample	*Mg. per 100 ml.*			Av.	Deviation %	Deviation %
1	2.90	2.75	2.85	2.83	+2.4	−2.9
2	3.20	3.20	3.35	3.25	+3.1	−1.5
3	3.15	3.20	2.95	3.10	+3.2	−4.8
4	2.80	2.85	..	2.83	+0.7	−1.0
5	3.25	3.35	3.35	3.32	+0.9	−2.2
6	3.55	3.65	3.75	3.65	+2.7	−2.7
7	3.65	3.90	3.80	3.78	+3.2	−3.4
8	4.25	4.30	..	4.28	+0.5	−0.7
9	4.55	4.65	..	4.60	+1.1	−1.1
10	3.30	3.15	..	3.23	+2.2	−2.5
11	4.80	4.40	4.55	4.58	+4.8	−4.0
12	4.15	3.75	3.90	3.93	+5.5	−4.6
13	3.10	3.00	3.25	3.12	+4.2	−3.8
14	3.10	3.40	3.40	3.30	+3.0	−6.1
15	3.50	3.60	3.55	3.55	+1.4	−1.4
16	3.25	3.40	3.10	3.25	+4.6	−4.6

Table II presents the individual values, average values, and per cent deviation obtained on 16 normal blood samples run in regular routine practice. The values range from 2.83 to 4.60 mg. of magnesium per 100 ml. of whole blood, which agrees very well with the most reliable values reported in the literature.

Work is now in progress on the analysis of a large number of blood samples from patients having malignant diseases to determine whether or not there is a correlation between the amount of magnesium present in the blood and the progress of the disease which would be of diagnostic importance. A similar study on a variety of diseases was undertaken by Zimmer (*16*), in which no correlation was obtained. However, this lack of correlation may be due to the fact that consistently high results with an average precision of ±13 per cent were obtained by the spectrographic method which she used.

Acknowledgment

The author wishes to express her appreciation for the very fine cooperation and assistance given by the members of the X-Ray Department at Harper Hospital, and to the General Motors Corporation for free license to Patent 1,979,964.

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Determination of Ammoniacal and Nitrate Nitrogen in Decomposed Plant Material

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In studies on the preferential utilization of different forms of nitrogen during the decomposition of plant materials (*5*) the inadequacy of existing methods was apparent. This aspect of the problem of decomposition arises in connection with the practice of incorporating into the soil fresh undecomposed farmyard manure saturated with urine, or, less frequently, straw supplemented by a dressing of ammonium sulfate. In such circumstances, in addition to the ammonia, nitrogen may also be available in the form of soil nitrate. Subsequent changes in the amounts of the various forms of nitrogen were hard to follow, especially when the quantities involved were small, because of inaccuracies in existing procedures when applied to decomposing or decomposed residues and manures.

The common method of estimating ammoniacal and nitrate nitrogen in decomposed vegetable material is that of distillation in presence of magnesium oxide for ammonia, and subsequent reduction of the residue with Devarda's alloy for nitrate. The conditions under which such estimations are made are drastic and some of the ammonia, which is liberated at the abnormally high pH of 10 to 11 produced by the use of magnesia, comes from the plant amides. The results thus obtained are apparently higher than the true ammonia or nitrate content of the samples under examination. Another method which is sometimes employed is the extraction of the residues or manure with sodium chloride solution, thus liberating the ammonium ion by the process of base exchange, and subsequent distillation of this extract with magnesia. This method, being laborious and time-consuming, is still unsatisfactory, as some of the organic nitrogen may be dissolved out

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by the salt solution and hydrolyzed to ammonia at the high pH, giving higher quantities of ammonia and nitrate than are actually present. It thus is evident that the chief defect of the distillation method lies essentially in the use of magnesium oxide, which produces a highly alkaline reaction conducive to the hydrolysis of some of the organic nitrogen. The ammonia so liberated increases the apparent ammonia content of the sample.

Experimental

Ammonia. Nichols and Foote (*3*) showed that ammonia determinations in sewage and nitrogenous trade wastes were unsatisfactory if carried out by distillation with magnesia or 10 per cent sodium carbonate, because of the high alkalinity, and proposed the use of a phosphate buffer of pH 7.4 which causes no liberation of ammonia from such organic compounds as asparagine, acetamide, glycine, and arginine. Decomposing straw was accordingly distilled in the presence of amounts of phosphate buffers, giving four different reactions. The straw had been allowed to decompose without additional nitrogen having been supplied, and the high positive "nitrogen factor" of the straw (*4*) therefore precluded any possibility of ammonia accumulation in the decomposing residue. To this were added in each case 10 mg. of ammonia just prior to distillation (Table I). These results leave no doubt that the high alkalinity of the magnesia causes high ammonia figures to be obtained, and that by the use of a phosphate buffer at pH 7.4 this disturbing factor can be largely eliminated. Additional proof was provided by examining the effect of distilling 5 grams of undecomposed paddy straw (0.78 per cent nitrogen) with similar amounts of phosphate buffer and magnesia. Progressively increasing amounts of ammonia were obtained as the alkalinity was increased, no doubt as a result of hydrolytic deamination of organic nitrogenous constituents (Table II).

Table I. Recovery of Ammonia Added to Decomposed Straw

(10 mg. supplied in each case)

pH	NH_3 *Mg.*
7.0 (phosphate buffer)	9.50
7.4 (phosphate buffer)	10.11
8.0 (phosphate buffer)	10.84
8.5 (phosphate buffer)	11.65
10 (magnesia)	11.96

Table II. Effect of Concentration of Distilling Reagents on Hydrolysis of Nitrogenous Constituents of Air-Dried Paddy Straw

Phosphate Buffer *Cc.*	NH_3 *Mg./5 g.*	MgO *Grams*	NH_3 *Mg./5 g.*
40	0.00	4	0.70
50	0.00	6	0.84
60	0.00	8	0.98
70	0.00	10	1.01

The procedure finally adopted consists of distilling about 10 grams of moist residue or manure with 30 ml. of phosphate buffer in 300 ml. of distilled water. The buffer solution is prepared as described by Nichols and Foote (*3*) by dissolving 14.3 grams of KH_2PO_4 and 91 grams of K_2HPO_4 in 1 liter of distilled water. In this way distillation is accomplished at pH 7.4 and the only precautions necessary are those to prevent undue frothing.

Nitrate. The cause of an apparently high yield of ammonia by the magnesia method is also operative in the nitrate determination, and accordingly a modification of the Bengtsson (*1*) procedure was employed.

Table III. Recovery of Nitrate Nitrogen Added to Manure

Nitrate Nitrogen Added *Mg.*	Nitrate Nitrogen Recovered *Mg.*	Recovery %
5	5.01	100.2
10	10.06	100.6
15	15.15	101.0
20	20.25	101.3

Table IV. Recovery of Ammonia and Nitrate Nitrogen from Decomposing Straw

(Comparison of old and new methods. Grams of nitrogen per 100 grams of original straw)

Days	Ammoniacal Nitrogen MgO	Ammoniacal Nitrogen Buffer	Nitrate Nitrogen Old method	Nitrate Nitrogen Modified method
	N Added as Ammonium Nitrate			
0	0.57	0.57	0.57	0.57
3	0.42	0.32	0.50	0.49
7	0.21	0.05	0.36	0.15
15	0.28	0.03	0.31	0.04
30	0.19	0.02	0.21	0.01
56	0.13	0.05[a]	0.19	0.03
	N Added as Ammonium Carbonate and Sodium Nitrate			
0	0.57	0.57	0.57	0.57
3	0.45	0.36	0.48	0.48
7	0.25	0.04	0.35	0.12
15	0.28	0.03	0.30	0.03
30	0.21	0.02	0.22	0.01
56	0.14	0.04[a]	0.17	0.02

[a] Increase due to ammonification of fungal protein which sets in after about 6 weeks' decomposition.

Ten grams of the fresh sample are extracted with 300 ml. of distilled water in 50-ml. portions. Each fraction remains in contact with the sample for about 10 minutes prior to filtration through a cotton plug. Extraction is continued until no blue coloration is obtained with diphenyl benzidine, a test sensitive to one part in a million (*2*). Suspended colloids are then precipitated by the addition of a few drops of sulfuric acid (20 ml. to 100 ml. of water). The solution is warmed and coagulated matter is removed by suction. By addition of caustic soda the filtrate is made alkaline, and boiled down to 30 ml. A little more caustic soda is added and the volume is made up to 200 ml. before concentrating again to 30 ml. In this way ammonia initially present as such, or capable of being liberated from the small amount of organic nitrogenous material present, is removed. The residue is finally distilled with Devarda's alloy after dilution to 300 ml.

The effect of this improved procedure on the recovery of nitrate nitrogen added to manure is shown in Tables III and IV.

Summary

The magnesia distillation method is unsuitable for the determination of ammonia in decomposed plant residues or manure, because high results are obtained through the concurrent liberation of ammonia from organic nitrogenous substances. The substitution of a phosphate buffer giving a reaction of pH 7.4 is recommended.

Nitrate nitrogen can be determined on the aqueous extract of such materials by the Devarda reduction method after removing free or liberated ammonia by boiling under alkaline conditions.

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Machine and Methods for Testing Mechanical Stability of Latex

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Methods and machines have been studied for making mechanical stability tests on latices. The results indicate that a constant-speed machine will give reproducible results at widely separated laboratories when test samples of fixed solids content are used.

THE mechanical stability of a sample is one of the important factors in processing latex. Therefore it is necessary to have a testing method which is simple and reasonably rapid yet gives accurate and reproducible results.

The various methods of testing mechanical stability where latex is used and tested result in a different standard at each place and create confusion when it is necessary to compare results obtained by different procedures.

This paper gives the results obtained with the original standard test and modifications which were introduced in an attempt to establish a universal and more accurate test for determining the mechanical stability. In this work three different methods were used and in each one two different types of stirrers were tried (Figures 1 and 2).

One machine, used as a high-speed stirrer, was a Benedict Indestructo mixer, No. 53, Serial No. 23270, 105 to 120 volts, 70 watts, and 25 to 60 cycles. The other machine was a constant-speed stirrer built at the Firestone Laboratories. It consists of a vertically mounted Bodine synchronous motor of 1800 r. p. m., 110 volts, 7.4 amperes, 0.2 horsepower, and 60 cycles, with a 20.15-cm. (8.06-inch) wooden pulley mounted on the motor shaft, and a rubber fiber belt connecting the pulley to a stainless steel shaft 2.5 cm. (1 inch) in diameter, mounted vertically in a steel tripod. The steel shaft is furnished with a metal propeller attached to the lower end, and this makes up the stirring element. The motor support and steel tripod are permanently mounted on a wooden base plate. This construction is not to be considered the best equipment that could be built, but it did serve its purpose for this work. Plans are being drawn up for a simpler and more permanent piece of apparatus.

FIGURE 1. BENEDICT STIRRER

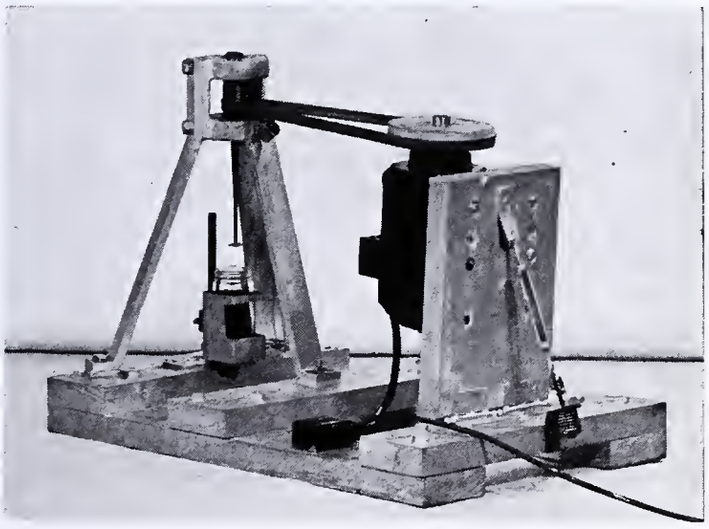

FIGURE 2. CONSTANT-SPEED STIRRER

Methods Used

Method 1A is carried out on the Benedict mixer. A 50-cc. sample of the latex is obtained from the drum and is stirred at 25° C. in a 118-ml. (4-ounce) square bottle with a high-speed stirrer until completely coagulated. The stirrer is equipped with a hexagonal, slotted propeller, as shown in Figure 3.

Method 1B is exactly the same as 1A, except that the test is carried out on the constant-speed stirrer, which is also equipped with the hexagonal slotted propeller.

Method 2A is a modification wherein an 80-cc. sample of latex with 50 per cent total solids and 0.5 per cent ammonia is held at 35° C. and is stirred in a 250-cc. round bottle with the Benedict mixer.

Method 2B is the same as 2A, except that the constant-speed stirrer is used.

Method 3A is a slight modification of Method 1A, using a sample diluted with distilled water to a total solids content of 50 per cent and 0.5 per cent ammonia. The Benedict mixer is used in this case.

Method 3B is the same as 3A, except that the constant-speed stirrer is employed.

FIGURE 3. PROPELLER

Speed Measurements

The shaft speed in r. p. m. of each stirrer was measured by the use of an Edgerton stroboscope under various operating conditions. This stroboscope was capable of measuring speeds directly, as shown on the scale, up to 3000 r. p. m. Beyond this point, factors had to be used when perfect synchronization was obtained, to calculate the shaft speed.

The constant-speed stirrer was operated in media of different viscosities. When running free, the stirring element of this machine ran at 13,420 r. p. m. On checking the same shaft in such media as water, concentrated latex, glycerol, and Karo sirup that had been cooled to 10° C. in order to make a very viscous medium, this speed remained constant without varying over 15-minute periods. In view of these results, there is no question as to the constant speed of this stirrer during any test.

The new Benedict mixer was tested with various latex samples of high total solids content for constancy of speed by means of the stroboscope. This machine was strongly affected by the loads under which it was running. Under load of a sample of latex of 60 per cent total solids, the speed varied from 17,000 to 20,000 r. p. m. during the actual test; after coagulation took place, the speed was reduced to a much lower value. In samples of total solids contents of 40 and 50 per cent, the speed variations were not very great. Although there was a considerable variation in the speed of the Benedict mixer under load, the variation over actual testing periods tended to average itself, and in some cases the speed was very close to 19,000 r. p. m. during the test. In the samples of latex having 40 and 50 per cent solids, the average was very good under what might be termed normal operating conditions. However, in many cases conditions were not normal, and in these cases the speed of the machine varied considerably and gave different results.

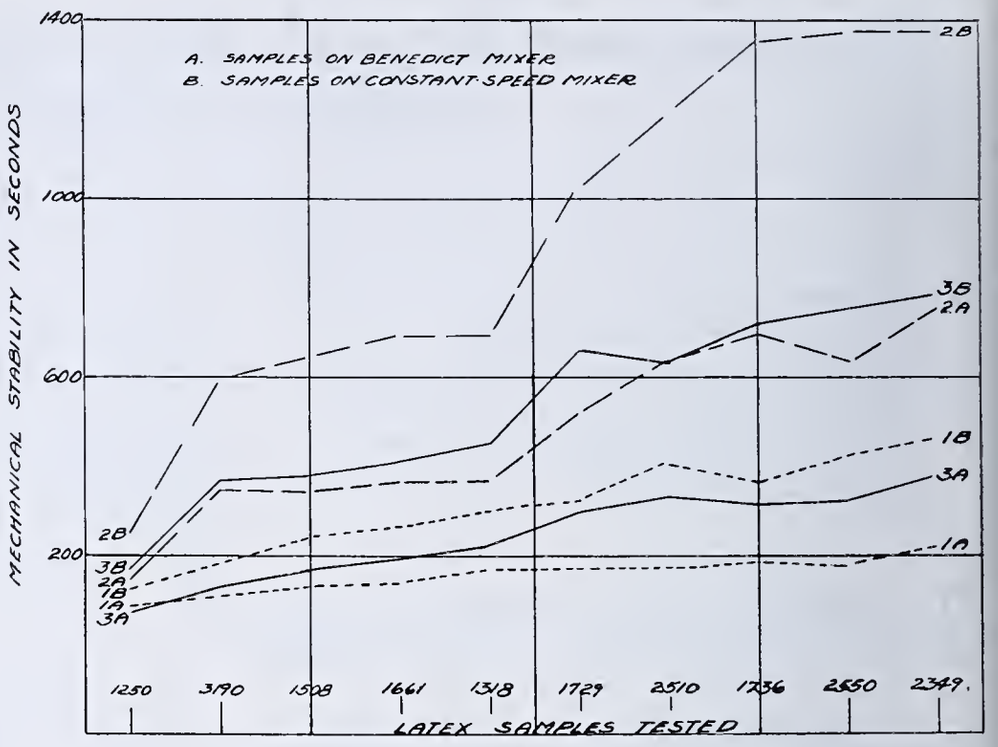

FIGURE 4

TABLE II. MECHANICAL STABILITY TESTS ON LATEX

	Methods 2B and 2A					
	Constant-Speed Mixer			Benedict Mixer		
Sample	Plant 1	Plant 2	Plant 3	Plant 1	Plant 2	Plant 3
1250	160	160	150	165	145	120
1661	520	530	530	540	475	240
1729	450	435	480	440	355	240
2349	480	480	460	550	440	255
3190	315	325	375	325	280	140

The above results indicate that the constant-speed stirrer is very reliable, and that results on the Benedict mixer averaged fairly well over a run, but in a good many tests the results were changed by the inability of the mixer to keep a constant r. p. m. This was due to change in operating conditions which must be expected when handling such materials as latex where coagulum is introduced during the test. If the coagulum comes into contact with the shaft in any way so as to create high friction and thereby a braking action, it upsets the value for this particular test.

TABLE I. DUPLICABILITY OF RESULTS IN DETERMINING MECHANICAL STABILITIES

Drum No.	Total Solids	Method 1A			Method 1B		
		Run 1	Run 2	Av.	Run 1	Run 2	Av.
	%						
1250	61.25	92	102	97	130	128	129
1318	60.91	175	166	171	306	295	301
1508	60.92	130	143	137	249	233	241
1661	60.82	128	155	142	253	274	264
1729	59.78	176	170	173	317	326	322
1736	61.42	180	186	183	357	360	359
2349	61.87	208	215	212.	436	463	450
2510	62.86	170	159	165	425	390	408
2550	62.81	180	161	171	423	419	421
3190	60.72	110	114	112	217	165	191
		Method 2A			Method 2B		
1250	...	151	135	143	260	251	256
1318	...	355	374	365	698	694	696
1508	...	352	337	345	646	640	643
1661	...	369	374	372	701	695	698
1729	...	529	522	526	1021	1017	1019
1736	...	710	687	699	1350	1339	1345
2349	...	728	760	744	1380	1352	1366
2510	...	627	645	636	1160	1176	1168
2550	...	625	639	632	1380	1358	1369
3190	...	343	355	349	585	583	584
		Method 3A			Method 3B		
1250	...	73	72	73	163	167	165
1318	...	249	210	230	457	442	450
1508	...	163	170	167	389	375	382
1661	...	185	196	191	413	401	407
1729	...	297	300	299	660	672	666
1736	...	313	300	307	704	713	709
2349	...	375	361	368	770	782	776
2510	...	331	326	329	632	626	629
2550	...	333	309	321	755	738	747
3190	...	129	123	126	393	343	368

Experimental Data

Ten samples of latices, selected over a period of one year to give as wide a range as possible, were taken for these tests. Each sample was run at least twice according to the methods described above, and the data obtained are given in Table I. The constant-speed stirrer gives the most accurate results in all cases. In this present setup, the constant-speed stirrer requires a longer time for end points in the test. Methods 1A and 1B give extremely rapid results; because of this, it is impossible to bring out small differences in stability between two samples. Methods 2A and 2B correct this somewhat, but still do not give the desired differentiation between samples. If, however, we consider methods 3A and 3B, there are larger differences between samples.

The results presented in Figure 4 and Table I in many cases hardly seem to show the necessity for using a constant-speed stirrer. However, in some cases it was necessary to run a third sample when using a Benedict stirrer because the original results were too far apart to average.

The following data are very convincing as to the need of a constant-speed stirrer in the latex industry. The Firestone Tire & Rubber Company has need for determining the mechanical stabilities of latices at four widely separated points. Even though the specifications for test were drawn up at the home office and equipment was purchased in each case from the same company under specifications and checked at the

home laboratories with the determination of factors if necessary, there was a good deal of confusion and inconsistency in the results obtained at the various places. These discrepancies brought about the work which is presented in this paper. The following results indicate difficulties which are very apt to be encountered with the use of the malted milk (Benedict) type of mixer.

The test results (Table II) were first obtained at the home laboratories with samples selected and prepared at that point. These results were not furnished to the subsidiary plants, but they in turn determined the stabilities, on both the Benedict mixer and the constant-speed instrument, of the samples which had been prepared at the home plant. Table II shows the wide discrepancies which can be encountered with what at first might seem to be a very reliable and accurate method of determining mechanical stabilities, but with which it seems practically impossible to duplicate results in different laboratories. Here the constant-speed stirrer has shown its reliability.

There probably will be need for further standardization of this test, but it can readily be seen that it merits adoption.

Tachometers or other speed-determining instruments may be attached to the Benedict mixer. In this way a record may be kept of the speed from time to time, an average may be made over a period of time, and a factor may be introduced for correcting the result in seconds. But the observation of the end point in any mechanical stability test requires the attention of the operator at all times. Also, any instrument which must be attached to the malted milk mixer for recording speed, etc., immediately removes its only advantage over the constant-speed mixer: cheapness. The cost of the constant-speed stirrer is slightly higher, but will be less than that of a Benedict mixer with all other instruments attached. The constant-speed stirrer is much simpler to operate and the test in itself is simpler, inasmuch as the operator has only the sample under test to consider.

A point which has not been studied in any detail in this investigation has been the type of propeller used on the stirring element. This should be given some consideration, as the propeller itself can affect the result if it is not readily self-cleaning when pieces of coagulum are picked up on the edges which cause the major portion of the agitation. A propeller should be adopted which can be easily reproduced in any machine shop and not have to be stamped out with a die.

The type of container, the quantity of latex, specifications as to solids content, and ammonia content are all very important in this test. However, the two factors of solids content and ammonia content need not be introduced into a standard specification if the equipment itself is standardized sufficiently to give reproducible results at different laboratories. If such specifications are followed, an ammonia content can be decided by the laboratories using this type of test.

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PRESENTED before the Division of Rubber Chemistry at the 99th Meeting of the American Chemical Society, Cincinnati, Ohio.

Silica and Pyrex Glass Stills with Automatic Constant-Pressure Feeds

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INVESTIGATIONS on the importance of small quantities of the heavy metals for plant growth and animal health have necessitated the production of water of greater purity than is usually required for analytical work. In the usual types of laboratory still significant amounts of some of the heavy metals may be carried over with the steam or derived from the materials used in the construction of the boiling chamber and the condenser. The ordinary distilled water available in these laboratories, although otherwise of very high quality, contains 13 to 40 micrograms of zinc and 20 to 30 micrograms of copper per liter. To obtain water with a lower content of heavy metals redistillation from silica or Pyrex glass is essential, and the production of small quantities presents no difficulties. When larger amounts are required, as for water culture experiments, some form of continuous distillation is necessary. Several stills for continuous operation have been described (*1–4*), but judging by the relatively complicated arrangements employed, it would seem that the very simple form of continuous feed from a constant-pressure Mariotte's bottle has been overlooked.

Three types of stills for continuous operation which have been used extensively in these laboratories are described below. These stills have been designed to yield from 1 to 2 liters of redistilled water per hour, and have been operated continuously for long periods. One still produced nearly 1000 liters of water per month for 4 months.

In all three stills a Mariotte's bottle maintains a constant level of water in the still. The aspirator bottle may be refilled, and the boiling flask drained, washed, and refilled, merely by the operation of the appropriate stopcocks. No dismantling of any part of the apparatus is necessary.

The supply of ordinary distilled water in the Mariotte's bottle is replenished by connecting the large-bore tube, *E* (11 mm. in diameter), in Figure 1, to a source of distilled water. Stopcocks *B* and *C* are closed, and stopcock *A* is connected to a slowly operating filter pump, giving a reduction of pressure in the bottle not greater than 25 to 30 cm. (10 to 12 inches) of mercury. When the bottle is full, stopcock *A* is closed and *B* is opened. Atmospheric pressure is thus established at the lower end of tube *B*. When stopcock *C* is opened the level of the water in the boiling flask, at atmospheric pressure, rises until it reaches the level of the lower end of tube *B*, also at atmospheric pressure. Air flows into the bottle through tube *B*. So long as the end of tube *E*, within the bottle, is lower than the outlet of tube *B*, no stopcock is required on it. (In laboratories where the supply of distilled water is under pressure, the method of refilling the Mariotte's bottle is essentially the same.) The boiling flask is drained and washed by operating stopcocks *C* and *D*.

Figure 1 (left) shows a still with double surface condenser constructed of Pyrex glass and arranged for gas heating. The flask has a capacity of 3 liters and all ground joints are interchangeable (standard size ₹29/42 with minimum inside diameter of 20 mm.). There is no constriction of bore at the joints or in the condenser. The minimum bore of 18 mm. prevents appreciable back pressure being developed in the flask and the level of the water is not lowered on vigorous boiling. While this arrangement is desirable, a

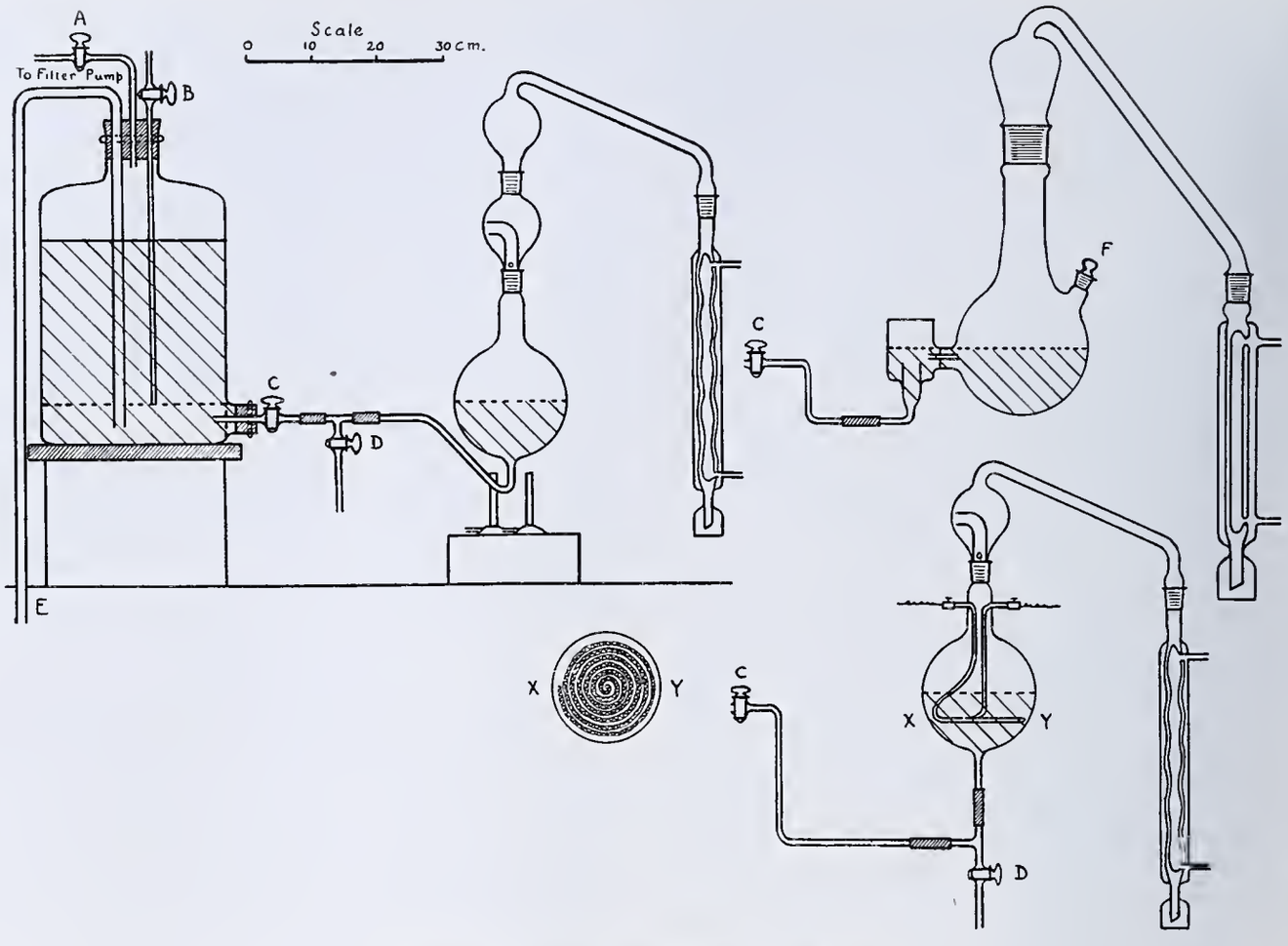

FIGURE 1. SILICA AND PYREX GLASS STILLS

small back pressure in no way affects the constancy of the automatic feed, once equilibrium is reached. It is only necessary to raise the level of the lower end of tube *B* to compensate for this back pressure.

Rubber comes into contact only with the cold water being fed into the still. The feed through the bottom of the flask has been found very satisfactory, since hot water cannot flow downward into this tube and no air locks are formed. Boiling is very even and free from bumping. The double splash head ensures freedom from spray even at high rates of distillation. This still will produce 2 liters of high-grade redistilled water per hour, but it is usually operated at 1.3 to 1.6 liters per hour. A trace of copper and other heavy metals can often be detected in the first 50 ml. of distillate each time the still is started, and it is therefore usual to reject the first 200 ml. of distillate. The redistilled water, as collected, contains not more than 0.2 to 0.3 microgram of copper per liter.

Figure 1 (lower right) shows a similar type of still, the unique feature being the immersion type of electric heater, *x*, *y*. A heating element constructed of a helix of Nichrome wire enclosed in a spiral of snugly fitting thin-walled Pyrex glass tubing combines the high efficiency of an immersion heater with freedom from metallic contamination. The spiral heating element is sealed into the flask after the latter has been cut near its maximum circumference. Heavy-gage copper wire is welded to the ends of the Nichrome wire to form cold leads to the terminals, which are mounted firmly, but not tightly, on the ends of the glass tubes projecting through the neck of the flask. Since the heating element is immersed in water, the Nichrome wire will carry a large current without a big rise in temperature. In the still as constructed 20 B & S gage Nichrome wire is used, and with a current of 6.3 amperes (202 volts, 1270 watts) the temperature of the wire is well below dull red heat. (Calculations show that the inside surface of the tubing is at a temperature of about 105° C. with the outside surface at 100° C.) To ensure a low temperature for the wire, the coils should fit closely against the inside surface of the glass tubing. The wall thickness of the tubing is of minor importance. The low temperature of the wire gives a practically indefinite life for the heating element. This type of immersion heater is suitable only for redistillation of water, since any scale formation, from ordinary water, would lead to overheating the glass tubing.

The high efficiency of the still, without any external thermal insulation, can be appreciated from the following figures: room temperature (temperature of feed water), 17.5° C.; power required for operation, 1270 watts; output of redistilled water, 1560 ml. per hour.

As 0.73 kilowatt-hour is required to raise the temperature of 1 liter of water from 17.5° to 100° C. and to vaporize it, the efficiency of the still is about 90 per cent. A higher room temperature gives a greater efficiency, due mainly to smaller radiation losses from boiling flask and splash trap. Redistilled water from this still has been found to contain total heavy metals of the order of 1 microgram per liter.

The presence of boron in Pyrex glass renders it unsuitable for some work and where freedom from this element is required stills can be constructed of fused silica.

Figure 1 (upper right) shows such a still, the boiling flask being made of translucent Vitreosil, while the splash head and double surface condenser are made of transparent fused silica. A stopper in the body of the flask allows the introduction of a siphon tube for draining the residue from the flask without dismantling. The cold-water feed into the flask is through a capillary tube of suitable bore connecting the feed chamber to the flask. This still is designed for gas or electrical heating. With a 1500-watt radiant type of heater and good thermal insulation, distillation is at the rate of 1.2 to 1.3 liters per hour.

Acknowledgment

Acknowledgment is made to V. A. Stephen, technician at the Waite Institute, who constructed the immersion heater (lower right) and sealed it into the boiling flask.

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A Gelometer for Starch Pastes

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SHEPPARD (*8*) and Bogue (*2*) have reviewed the methods for measuring jelly strength. These fall generally into two classes—those in which the jelly is broken, as in the Delaware jelly strength tester (*1, 11*), and those in which it is deformed only a specified amount, as exemplified by the Bloom gelometer (*4*). Neither type of measurement is entirely satisfactory because the breaking strength of a gel is not always an indication of its resistance to deformation (*7*).

Consequently, there has come into favor a third class of instrument which takes both these properties into consideration. Thus, the methods of Sheppard (*8*) and of Saxl (*5, 6, 7*) give a more complete picture of the rheological properties of a gel than was possible by the older methods. The apparatus described in this paper was designed especially for use with 6 to 9 per cent starch pastes, and will measure on them the same properties that Saxl's gelometer measures on more resistant materials.

Description of Apparatus

The gelometer shown in Figure 1 consists of a regulator, *B*, to give constant head of suction; expansion chamber, *C;* screw clamp, *D*, to regulate rate of flow of air through the system; water manometer, *E*, to read the amount of suction applied to the paste as determined by the height of water column in *H;* brass platform, *J*, with hole in center over which starch paste is placed; graduated pipet, *F*, for reading volume of water displaced by the gel as it is deformed by suction; and constant-head capillary-overflow, *K*, to provide constant rate of increase in suction applied to the paste.

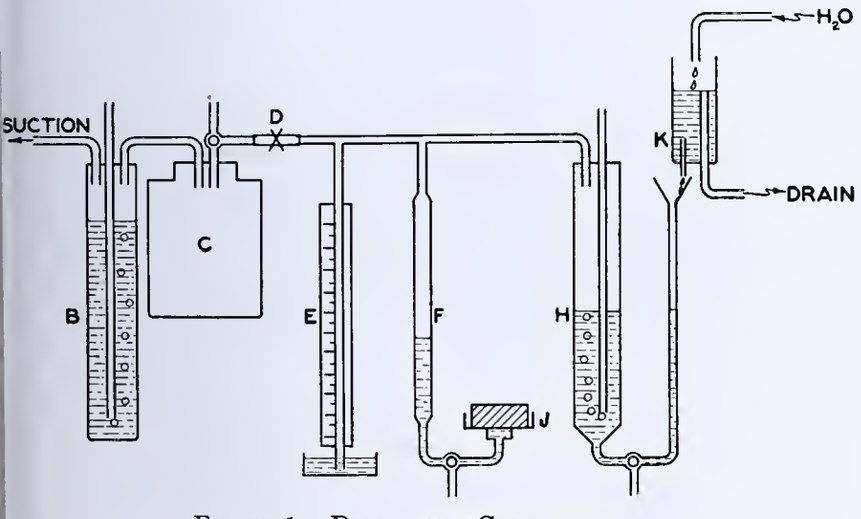

FIGURE 1. DIAGRAM OF GELOMETER

Procedure

The weighed sample mixed with 150 ml. of water is pasted by heating at 99° C. for 0.5 hour in a water bath. In order to prevent excessive evaporation, the tubes are covered by rubber stoppers containing a hole in which a stirring rod is placed. The pastes are stirred by hand from time to time to allow for uniformity in heating. Violent mechanical agitation is avoided, since it increases evaporation losses and may injure the granules. The paste is poured into a 400-ml. beaker, covered with a watch glass, and allowed to stand for 15 hours at room temperature. The gel may then be removed from the beaker and placed bottom down on the brass platform, *J*.

Water is turned on at *K* and suction applied. As water drips from the capillary into the funnel, the level in *H* rises at a constant rate. During this time, readings are taken on the manometer, *E*, and pipet, *F*. Rates of 1 to 4 cm. per minute increase in suction are convenient and allow ample time to make accurate readings. When plotted (Figure 2) the readings of cm. suction *vs.* ml. deformation show a straight-line relationship in the range where the gel is elastic. The breaking point of the gel is indicated by a fairly sharp break in this line.

In order to obtain hysteresis curves, the three-way stopcock below *H* is opened before suction has reached the elastic limit, and readings are made as the force on the gel is gradually reduced. From these three quantities—elasticity, breaking point, and hysteresis—may be calculated the gel factor, Bloom point, elastic recovery, and other values which have been used as expressions of gel strength (*5*).

The slope of the suction-deformation line (indicative of elasticity) is roughly proportional to the area of the hole over which the gel is placed. The amount of suction (centimeters of water) necessary to break the gel, since it is exerted equally on each unit area, is relatively independent of hole size except in cases where the gel is deformed by its own weight.

Starch pastes are extremely sensitive to variations in the method of preparation; before starting routine measurements on this instrument, it is essential that the method of preparation be checked as to duplicability of the product.

Principles

The combination of principles which differentiates this gelometer from others described in the literature may be summarized as follows:

1. Suction is used to deform the gel (*9*). The force is thus transmitted uniformly to all parts of the gel exposed to it. Since the deformed portion is in contact only with water, such factors as abrasion due to mechanical plungers and inconveniences due to the use of elastic membranes are eliminated.
2. The volume of deformation is measured by hydrostatic means (*10*). Using a hole size of 2.6-cm. diameter and a 2-ml. pipet, this volume may easily be measured to 0.02 ml., corresponding to a deformation of 0.04 mm. by instruments which measure depth directly.
3. The deforming force changes slowly, uniformly, and at a constant rate. Having started the flow of water from *K*, the operator may

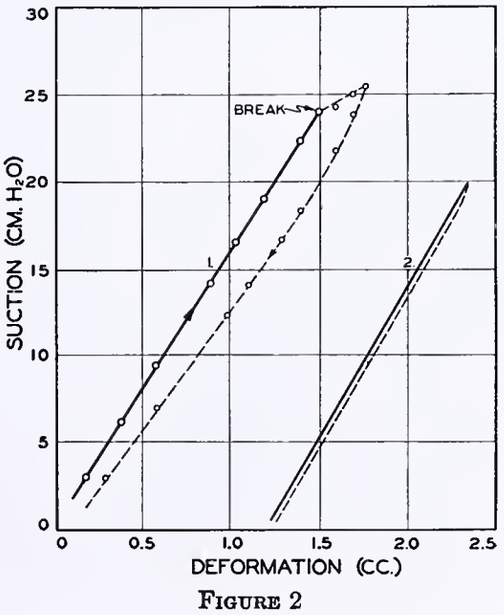

FIGURE 2

Table I. Comparison of Breaking Strength and Elasticity of 6.5 Per Cent Starch Gels

Starch	Breaking Point *Cm. H_2O*	Slope *Cm./ml.*	Rigidity × 10^{-1} *Dynes/sq. cm.*
Yellow Creole	18.8	15.0	660
Popcorn	17.5	13.8	590
Mandan	12.5	10.7	440
Commercial corn	10.5	11.0	370
Hegari sorghum	7.8	10.3	378
Modified A	9.5	9.5	320
Modified B	7.0	9.0	270
Modified C	4.5	7.5	205
Country Gentleman	3.5	7.5	175

concentrate on reading the manometer and pipet with no further attention to the mechanics of increasing the load. This uniform change of applied force is especially valuable in measuring properties where the time factor is important and stepwise addition of load would be likely to introduce error.

4. Skin effects and the influence of the walls of the containing vessel are eliminated by removing the mold from the beaker and placing it bottom down on the brass platform. The dimensions of the gel in relation to the area to which suction is applied should be large enough so that deformation occurs chiefly in the part adjacent to the hole without causing the rest of the mold to change its shape appreciably.

Applications

Figure 2 indicates the type of information which may be obtained on this apparatus. Curve 1 is typical for starch pastes. The amount of deformation proceeds in direct proportion to the increasing suction up to the point where the gel breaks, with a corresponding sharp break in the straight line (at 23.5 cm. in this case). Such behavior tends to show that starch gels of this concentration (7 per cent) are almost perfectly elastic, since they do not undergo a period of yield before actual shearing; otherwise the straight-line relationship would not hold at pressures close to the breaking point. The fact that the gel is broken may further be confirmed by direct observation upon removing the mold from the platform. A circular cut is usually produced; sometimes a hemispherical piece is torn from the center.

The dotted portion of curve 1, representing gradual reduction of suction, has no significance since the gel has been broken. It is included for comparison with curve 2 where suction on the paste was increased up to 20 cm. (just below the breaking point) and then reduced to its original value.

The difference between the ascending and descending portions of curve 2 (0.05 ml.) indicates that hysteresis is present to only a limited extent, about 2 per cent of the total deformation. Further evidence supporting this view was obtained by subjecting a starch paste with breaking point at 18 cm. to a constant suction of 16 cm. for 3 hours. The paste yielded only 0.01 ml. (or 0.002 cm.) per hour, which is probably beyond the range of accuracy of the apparatus.

Typical results obtained on starch pastes using this instrument are listed in Table I. The figures in the last column represent rigidities determined by the method described in a previous publication (*3*).

Both rigidity and slope are a measure of the force required to deform or stretch the gel a given amount. This relationship is evident from Figure 3. Rigidity values show greater differentiation, and when the concentration of paste can be kept below 6.5 per cent the rigidometer method is to be preferred. However, with concentrations of 6.5 to 8.5 per cent, rigidity determinations present difficulty because of the high consistency of the paste. It is in this range that the gelometer is useful, not only for values of slope, but for breaking point data.

This paper deals primarily with raw starches and slightly modified products. With more highly converted starches, the method of preparation would have to be altered by increasing the concentration and time of setting. Such gels would be expected to exhibit plasticity and hysteresis as the ratio of gelling to nongelling components becomes increasingly large.

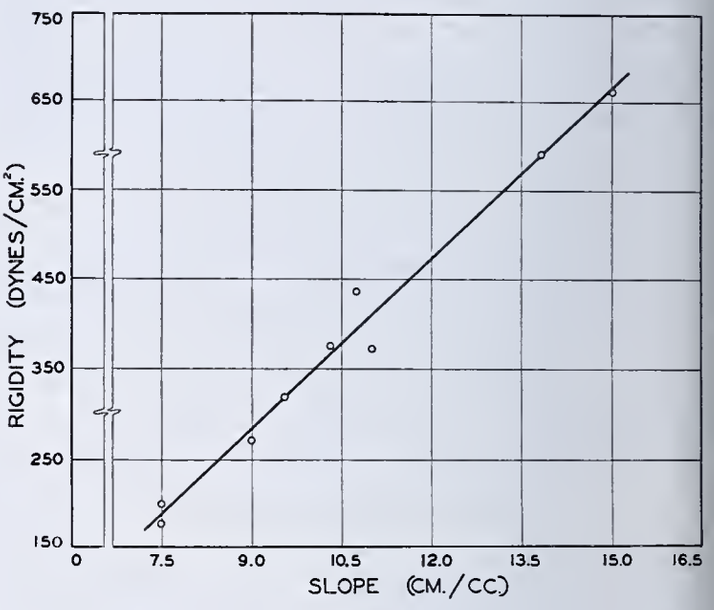

Figure 3

Summary

A gelometer for measuring elasticity and breaking strength of starch gels embodies the following principles: Suction is used to deform the gel, the volume of deformation being measured by hydrostatic means. The deforming force changes uniformly and at a constant rate. Skin effects and the influence of containing walls are eliminated. This apparatus is suited to starch concentrations of 6.5 to 8.5 per cent. For lower concentrations, the rigidometer described previously (*3*) gives greater differentiation with respect to elasticity.

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Correction. In the article on "Determination of Bromine Addition Number" [Ind. Eng. Chem., Anal. Ed., 13, 91 (1941)], the second line of the procedure, second column, should read "into a 25-ml. Erlenmeyer flask".

Karl Uhrig

An Improved Thixotrometer

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THIS thixotrometer is an improved design of one that was described in connection with a study of the leveling of paints (*2*). Apparatus of somewhat similar construction has been used by Schofield and Scott Blair (*3*) and by Freundlich and Röder (*1*).

To obtain better insulation from building vibration, the thixotrometer is now mounted on a pair of slings (Figure 1). Rubber bands suspend it so that the base just touches four rubber balls, placed under the corners of the base and flattened on one side to prevent their rolling. The balls furnish a damping effect and prevent swaying of the thixotrometer. The insulation from building vibration is very satisfactory.

To reduce to a minimum the stirring which resulted from the insertion of the sphere (*2*) into the material being examined, several changes were made (Figure 2). The torsion wire assembly was mounted on a precision rack and pinion (Pillor rack and pinion unit, Spencer Lens Company), the sphere was replaced by a steel disk of the same diameter, an electromagnetic clamp to hold the disk prior to release was constructed, and extension arms were made to permit operation of the micrometer (*2*) and the rack and pinion without jarring the thixotrometer. (Two mechanical clamps which were tried before resorting to the electromagnetic clamp were unsatisfactory. A wooden core is used in the electromagnet, since an iron core becomes permanently magnetized. Because alternating current causes a stirring action, direct current must be used in the electromagnet.)

To overcome any irregularities which might result from variations in filling the container (*2*), a funnel was constructed to permit filling while the container was in position in the water bath (*2*), and a vertical tube was mounted at the end of the container so that the source of filling would always be at the same point. This method of filling can be used only with thin materials, and so far no advantage in its use has been demonstrated.

To use the thixotrometer, the torsion wire assembly is raised, the torsion setting is made, and the disk is clamped in position by turning on the electromagnet. A stop watch is started the moment that the pouring of the material into the container is started. The disk is lowered into the material at the end of 1 minute and the disk is released at exactly 10 minutes. The distance traveled by the disk in 5 minutes is recorded.

The true zero position of the disk is that at which buoyant effects do not alter its position. The zero torsion setting is that which gives the true zero position of the disk. In order to keep the disk clear of the clamp, the clamped position is chosen 0.015 inch (0.04 cm.) behind the true zero position. If the distance traveled by the disk is less than 0.015 inch, the distance is determined by noting the initial and final positions on the eyepiece micrometer scale of the microscope. For greater distances of travel the disk is brought back to the true zero position by means of the micrometer and the correction of 0.015 inch is added to the micrometer reading.

The data in Table I were obtained in a series of consecutive measurements on a typical paint. $D_{obs.}$ is the distance traveled by the disk in 5 minutes for a torsion setting of 50°. $D_{calc.}$ is the corresponding value obtained from a line fitted to the data by the method of least squares. (In fitting the line the number of the observation was chosen as the abscissa and the corresponding distance of travel as the ordinate for the plotted points.) In the last column the per cent differences of the calculated from the observed values are given.

TABLE I. MEASUREMENT OF PAINT

No.	$D_{obs.}$	$D_{calc.}$	$\frac{100(D_{obs.} - D_{calc.})}{D_{calc.}}$
1	0.1229	0.1120	+9.75
2	0.1076	0.1140	−5.61
3	0.1075	0.1161	−7.40
4	0.1173	0.1181	−0.68
5	0.1215	0.1202	+1.08
6	0.1242	0.1222	+1.61
7	0.1247	0.1243	+0.32
8	0.1276	0.1263	+1.32

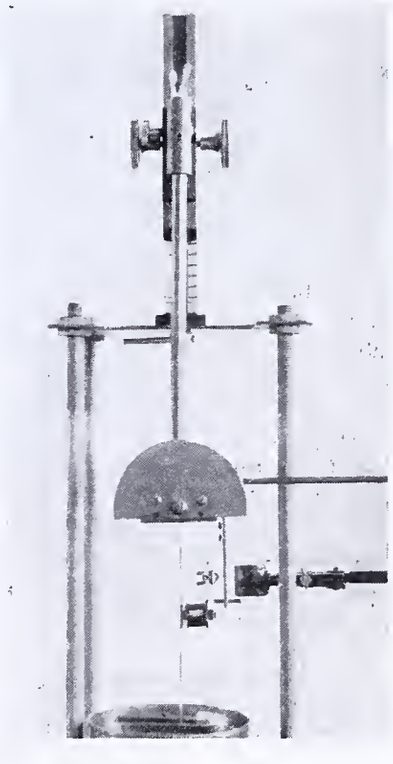

FIGURE 2. RACK AND PINION, AND ELECTROMAGNETIC CLAMP

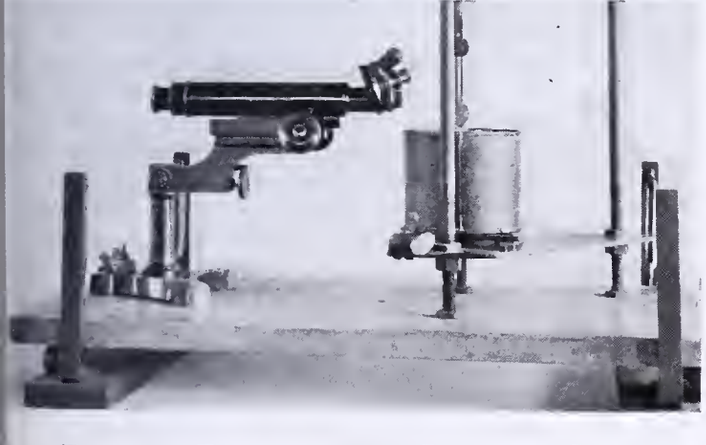

FIGURE 1. THIXOTROMETER IN POSITION ON SLINGS

From the differences in per cent the probable error of a single observation is found to be 3.49 per cent. The probable error of a single observation calculated from the results of 55 pairs of observations on various paints was found to be 3.55 per cent, which is in good agreement with the above value. These values seem large, but since variations of the order of a hundredfold are frequently encountered in comparing one paint with another, the precision of measurement is believed to be satisfactory.

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Application of the Grating Microspectrograph

To the Problem of Identifying Organic Compounds

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THE study of the optical properties of crystals has relatively greater importance in microscopic methods of identifying organic compounds than in the identification of inorganic ones. This is partly because with inorganic compounds chemical reactions of both cation and anion may usually be carried out under the microscope, whereas with organic compounds chemical tests are restricted to detecting the presence of certain reactive groups. However, in general, it is not possible to use optical crystallographic methods as the sole means of identification of organic compounds, because the optical constants have been determined for relatively few of the vast number of such known compounds. When, however, the optical data of organic compounds are used in conjunction with other physical and chemical data, they may be of considerable use to the microchemist, particularly where the problem may be reduced to that of finding out if an unknown substance is identical with one of a group of known substances.

The relative usefulness of various optical properties of organic compounds for such comparison purposes may be judged from the following considerations.

Principal Refractive Indices

It is convenient to follow the classification given by Wooster (*8*) and to divide molecular crystals into three main classes:

1. Almost spherical molecules, such as pentaerythritol and some of its esters. Crystals of this class have refractive indices well within the range of organic immersion media. Mixtures of Nujol with α-bromonaphthalene or α-iodonaphthalene have a sufficiently low solvent action to be used as immersion media.
2. Rod-shaped molecules. This classification includes the majority of long-chained aliphatic compounds. Usually the refractive indices of crystals of this class are low, unless the molecule contains substituents of high refractivity, such as bromine, iodine, sulfur, etc. The crystals may have an appreciable solubility in immersion media, so that refractive index measurements are not as a rule easy to make. Increasing the length of carbon chain by one or two carbon atoms does not greatly affect the refractive indices, and as the limit of accuracy of determining these refractive indices is about ± 0.002, it follows that the measurements may have but little analytical value.
3. Planar molecules. This classification includes the majority of aromatic compounds. Unless the molecules are grouped in nonparallel directions, n_γ and often n_β are beyond the range of known immersion media. Solubility of the crystal in immersion media may render it impossible to determine its refractive indices. The position and nature of weakly polar or nonpolar substituents usually have little effect on the refractive indices.

With all three classes of molecular crystal, n_α is usually low enough to be determined by the immersion method, using the light from a sodium vapor lamp for the final adjustment of the refractive index of the immersion liquid. The temperature variation method is not often applicable to organic compounds on account of their solubility in liquids having a suitably high thermal variation of refractive index. The author prefers to use the method of mixing two liquids of very low volatility and widely different refractive index on the microscope slide until the lowest refractive index of the crystal under test is matched. The immersion liquid is then transferred to a microrefractometer or an Abbe refractometer. Mixtures of tri-*N*-butyl citrate and *n*-butyl phthalate cover the range 1.447 to 1.492, and of Nujol and α-iodonaphthalene, cover the range 1.484 to 1.701 (*2*). Kunz and Spulnik have suggested the use of mixtures of heptylic acid and α-bromonaphthalene to cover the range 1.423 to 1.658 (*6*).

Birefringence

The birefringence of a doubly refracting crystal may have any value between the limits of zero for rays traveling along an optic axis, and $n_\gamma - n_\alpha$ (or $n_\omega \sim n_\epsilon$ in the case of uniaxial crystals) for rays traveling normal to the plane of the optic axis or axes. Consequently it is usual to restrict the term to the three constants $n_\gamma - n_\beta$, $n_\beta - n_\alpha$, and $n_\gamma - n_\alpha$ of biaxial crystals, and $n_\omega \sim n_\epsilon$ of uniaxial ones.

The birefringence of organic compounds is due to two factors, molecular anisotropy and structural anisotropy, and in general $n_\gamma - n_\alpha$ is large for rod-shaped or planar molecules which are oriented in the same direction, and is small for roughly spherical molecules and for planar molecules which have several orientations in the crystal. It may also be small for crystals containing solvent of crystallization, particularly when the solvent of crystallization also has planar molecules.

Methods of measuring birefringence with the aid of compensators entail measuring the thickness of the crystal, and as such thickness measurements can be made only with a low order of accuracy, the birefringences so determined are not very accurate.

Dispersion of Birefringence

The dispersion of refraction, $dn/d\lambda$, of a crystal is usually different for each of its principal refractive indices, and as birefringence is the numerical difference between two such

indices, it follows that birefringence varies considerably with wave length. So-called "anomalous" interference colors are caused by extreme dispersion of birefringence, but in no known case does the scale of interference colors given by a crystal exactly correspond to Newton's scale of colors given by an air film. Crystals which are pleochroic in the violet or near ultraviolet show marked differences between the dispersions of the principal refractive indices, and consequently have a strong dispersion of the birefringence, particularly along the acute bisectrix when the optic axial angle is small. By way of an example, the values of $n_\gamma - n_\beta$ of ammonium picrate and *o*-dihydroxybenzene are compared for various wave lengths.

The measurement of dispersion of the principal birefringences is complicated in the case of monoclinic and triclinic crystals which possess an appreciable degree of dispersion of the axes of the optical ellipsoid. In such cases the author prefers to measure the dispersion of birefringence presented by a fixed orientation of the crystal, and to take care that an identical orientation is used in comparisons with other crystals. Whenever possible, dispersion of the maximum birefringence, $n_\gamma - n_\alpha$, is chosen for comparison purposes, as errors of up to 10° in orientation of the crystal do not seriously affect the accuracy of the results. The measurements have considerable practical interest, as it is not necessary to know the thickness of a crystal in order to determine its dispersion of birefringence.

TABLE I. $(n_\gamma - n_\beta)$ OF A COLORLESS AND A YELLOW CRYSTAL

λ, mμ	*o*-Dihydroxybenzene	Ammonium Picrate
513	0.0106	0.044
541	0.0104	0.000
589	0.0099	−0.037[a]
656	0.0093	−0.071[a]

[a] Vibration directions of n_γ and n_β interchange at 541 mμ.

Optic Axial Angle

Observations of the interference figure given by a crystal in convergent polarized light have a well-recognized value in determining whether a crystal is uniaxial or biaxial, and, in the latter case, it is sometimes possible to measure the optic axial angle. Observation of the type of dispersion of the optic axes, when it can be made, renders possible differentiation among rhombic, monoclinic, and triclinic crystal symmetry.

Principal Absorptions

Observations of the principal absorptions of strongly colored crystals are usually qualitative and are confined to the study of dichroism or trichroism. This is understandable, as a great many strongly absorbing organic compounds form monoclinic or triclinic crystals which often have extreme dispersion of the axes of the optical ellipsoid, so that the optical properties of the crystals are not easy to interpret. Nevertheless, it is this complication of optical properties of absorbing crystals which makes them all the more specific and useful for comparison purposes.

The above considerations show the importance, from a determinative standpoint, of studying the optical properties of organic crystals for as wide a region of the spectrum as possible. It is obviously not practicable to repeat a complicated series of observations for many wave lengths, particularly as the eye is neither very sensitive nor very well focused for the extreme blue end of the spectrum.

Grating Microspectrograph

It was in order to simplify the study of crystal optics throughout the visible region of the spectrum that the author turned to the aid of photography and developed a transmission grating microspectrograph for use with the petrographic microscope. The first model of the apparatus used 8.125 × 10.625 cm. (3.25 × 4.25 inch) panchromatic cut film (*5*); the second model was much more compact and used 35-mm. Panatomic film (*3*). In neither of these instruments was the light collimated before its passage through the grating. The achromatic lens which projected the image of the slit onto the film had a very small diameter relative to the projection distance; the astigmatism thereby introduced into the spectrum was not at first considered serious. As experience in applying the apparatus to specific problems was accumulated, it became evident that it would be advantageous to have a spectrum completely free from astigmatism.

The generous gift of a plane metal grating from H. D. Babcock of Mt. Wilson Observatory made it possible for the author to construct a reflection-grating microspectrograph working on a somewhat different principle, which incorporated the improvements suggested by the earlier work. The general principle of the new apparatus is shown in Figure 1.

A Steinheil lens is used to focus the microscope image on the slit. The microscope, which is used with an eyepiece not fitted with cross webs, is focused for an image distance at infinity, so that the Steinheil lens is used at its principal focus from the slit. It is provided with centering screws, in order to bring the center of rotation of the image on the center of the slit. The collimating lens is a cemented achromat which collects light from the slit and gives a collimated beam which is then reflected by a silvered right-angled prism on the plane grating. A Wollaston prism, which is provided with a fine adjustment to its rotation, may be swung into the beam for studies of dichroism. Over half the light incident on the grating is diffracted in one of the first-order spectra, which is collected by a cemented achromatic camera lens and is brought to a focus in the image plane of a reflex camera which uses 35-mm. Panatomic film. It was found that much less coma was obtained when the plane side of the camera lens faced the grating and that all the spectrum was sharply in focus at the same time.

The grating is mounted in a heavily built holder which rotates on spindles fitted with Hoffmann ball bearings. The grating is rotated by a micrometer fitted with an accurately worked face plate of sapphire which presses against a steel ball fixed in the grating housing. In the center of the reflex ground glass, there is a clear patch fitted with adjustable cross webs. The spectrum can be focused on these cross webs which are viewed with a × 12 Ramsden ocular. Wave lengths of emission lines may thus be determined to 0.2 mμ. Spectra of very low intensity are observed through a telescope fitted with a reflecting prism which intercepts the light from the grating before it reaches the camera lens.

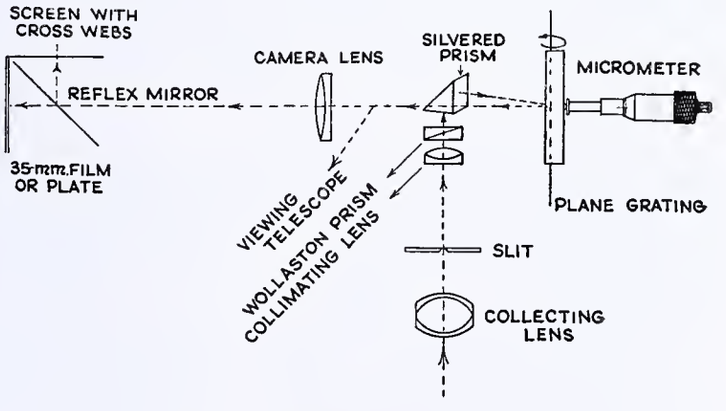

FIGURE 1. DIAGRAM OF APPARATUS

Compared with the transmission grating apparatus described by Chamot and Mason (*1*), the new apparatus is considerably easier to manipulate, as it is merely necessary to place the microscope under the microspectrograph. Accurate centration is not necessary; the microscope is moved on the baseboard until the spectrum is evenly illuminated, a matter of a few seconds' adjustment.

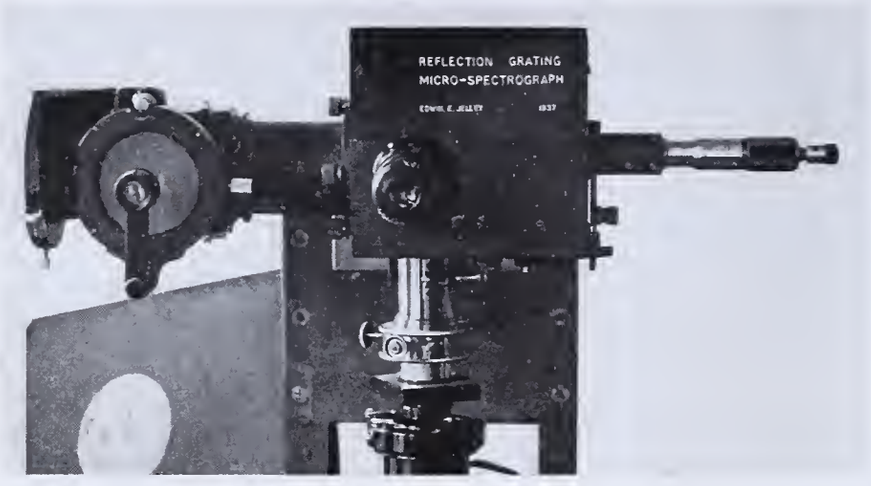

FIGURE 2. REFLECTION-GRATING MICROSPECTROGRAPH

The reflection-grating microspectrograph is shown in Figure 2, and the complete setup, with a Leitz universal polarizing microscope constructed to the author's specification, is shown in Figure 3. A silvered right-angled prism is used over the eyepiece to deflect an image of the crystal onto a screen. When the spectra are to be recorded photographically, the prism is thrown out of the optical axis of the system. There is, however, a disadvantage attendant on the use of a metal reflection grating; the violet end of the spectrum is relatively weaker than that obtained with a transmission grating. This is because the speculum metal on which the grating is ruled has a lower reflectivity in the violet, whereas the violet end of the spectrum is accentuated with a transmission grating by virtue of the higher refractive index in this region of the cellulose nitrate from which it is made.

Applications of Grating Microspectrograph

In the author's first publication on the microspectrograph (*5*), its use in the determination of optic axial angles of orthorhombic crystals for the visible spectrum was discussed. It was proposed to use a calcite plate cut perpendicular to the optic axis for calibration purposes. This plate was calibrated with sodium light ($\lambda = 589.3$) by means of a universal stage. In the second publication (*3*), a spectrogram was given which showed the dispersion of the optic axes of *o*-nitroacetanilide. The use of the microspectrograph in conjunction with a universal stage in determining the dispersion of a single axis of praseodymium sulfate octahydrate was also discussed. These publications also dealt with the "wedge-crystal" method of determining dispersion of birefringence.

Three microspectrographic methods of measuring birefringence and its dispersion as a function of wave length have been worked out.

DISPERSION OF BIREFRINGENCE OF WEDGE CRYSTALS. This is applicable to substances having a melting point (without decomposition!) below 300° C.

On an optically worked microscope slide of fused quartz is placed a square of No. 2 or No. 3 cover glass. Another square is placed so that one edge rests on the first square, with the opposite edge resting on the microscope slide. Some crystals of the substance to be studied are placed near the second cover slip, and the preparation is heated over a microburner until the substance melts and is drawn under the sloping cover glass to form a wedge of liquid. On cooling, the substance crystallizes to form wedge-shaped crystals. Refusion may be necessary to get satisfactory results, and the preparation may need to be seeded by touching it with a needle which has been charged with crystals of the substance. Such a wedge preparation shows ascending orders of birefringence when examined with a low-power objective between crossed nicols. The image of the wedge-shaped crystals, which have a slope of about 2°, is thrown on the slit of the microspectrograph and is so oriented that the slit cuts through the ascending orders. Spectrograms are then made on Panatomic-X film, exposures of 1 to 20 seconds being given, according to the width of the slit.

A Philips tungsten arc lamp is used as a light source, as it gives, in addition to the continuous spectrum, neon lines which serve to locate the wave-length calibration. A ribbon-filament lamp can also be used, but in this case an additional exposure is made with the microscope illuminated by a sodium vapor lamp. The simplest way of studying the spectrograms is to enlarge them so that 20 mμ on the wave-length scale is 10 mm. on the bromide enlargement. It is not necessary to use nonshrinking bromide paper, as uniform shrinkage in no way affects the accuracy of computations made from the enlargements.

If the wedge-crystal spectrogram shows several interference bands, it is best to proceed according to Figure 4. A line is ruled through the spectrum in a position corresponding to the thick end of the wedge crystal. Irregularities in the crystal cause many lines to run through the spectrum, and one of these should be chosen as a guide for the ruled line, thereby avoiding errors due to distortion introduced by the camera lens. A wave-length scale is then attached to the enlargement, and the wave lengths λ_n, λ_{n+1}, λ_{n+2}..... at which the nth $(n + 1)$th, $(n + 2)$th..... interference bands cross the line are recorded. The value of n is readily counted if the extreme tip of the wedge appears in the spectrogram. The value of the birefringence for any wave length, λ_n, at which an interference band of the nth order intersects the line, is $n\lambda_n/d$, where d is the undetermined thickness of the wedge at the point corresponding to the ruled line. It is convenient to consider the birefringence at different wave lengths as multiples of the value for sodium light, a procedure whiche liminates the necessity of determining the thickness, d, of the crystal. The calculations are simplified if the line is ruled through the spectrogram at an ordinate where an interference band occurs at $\lambda = 589$ mμ. In the diagram, this is the seventh band, which

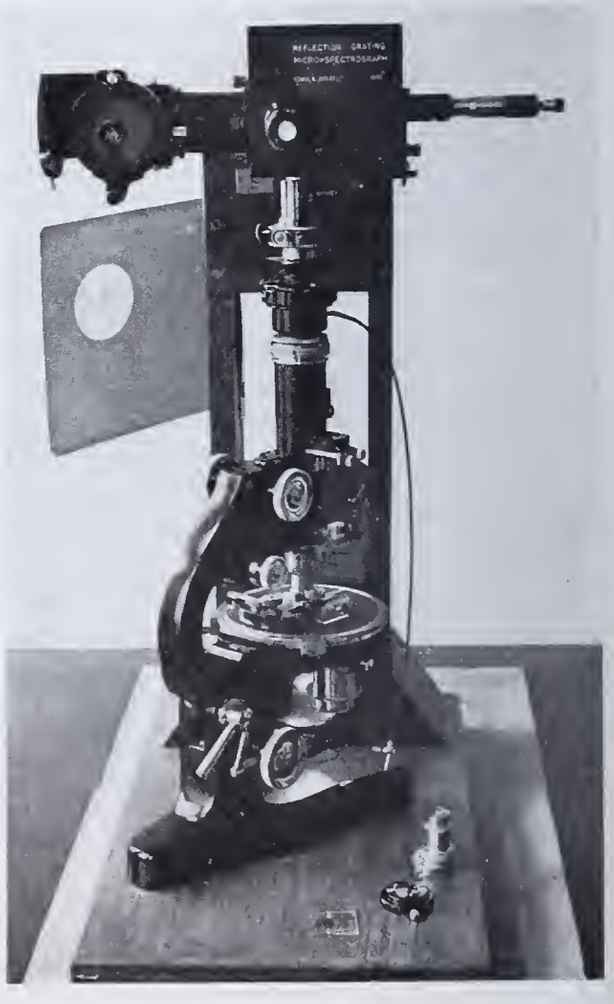

FIGURE 3. COMPLETE MICROSPECTROGRAPH SETUP

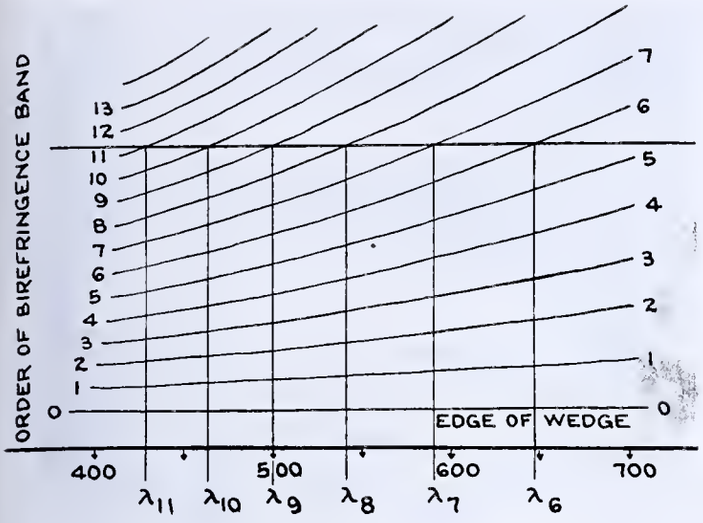

FIGURE 4. COMPUTATION OF BIREFRINGENCE

corresponds to a retardation of 589 mμ × 7 = 4.125μ. Similarly, the retardation at λ_6 is 647 mμ × 6 = 3.922, and at λ_9 is 500 mμ × 9 = 4.5μ (retardation = birefringence × thickness of crystal). The values of the relative birefringence are, therefore, 0.95 for 647 mμ, 1.00 for 589 mμ, 1.09 for 500 mμ, and so on. These values are plotted against wave length.

Some typical curves are given in Figure 5, in which Newton's color scale for an air film is represented by a horizontal line. This method of computing the dispersion of birefringence utilizes the light from a single point on the wedge crystal, and no assumptions are made as to the uniformity of slope of the wedge.

When, however, only a few bands are present, it is necessary to measure the average distance apart of the birefringence bands on the photographic enlargement at a number of wave lengths.

Let the average distance between bands at wave lengths λ_1, λ_2, λ_3..... be d_1, d_2, d_3..... The birefringence at these wave lengths is then represented by $c\lambda_1/d_1$; $c\lambda_2/d_2$; $c\lambda_3/d_3$..... where c is a constant depending on the slope of the crystal wedge and the magnification of its image on the bromide enlargement. If λ_1 is 589 mμ, the relative birefringence at λ_2 is $d_2.\lambda_1/d_1.\lambda_2$, and so on. These values are plotted against wave length, as above.

The value of the birefringence for sodium light may be determined with a moderate degree of accuracy by measuring the slope of the wedge on a goniometer or universal stage and measuring the distance along the wedge between the 2nd and 12th (or higher) interference bands when the microscope is illuminated with a sodium vapor lamp. If the distance between the 2nd and 12th bands, measured with a screw micrometer eyepiece, is $d_{mm.}$ and the slope θ, the birefringence is $0.00589/d \tan \theta$. The value of the birefringence for any other wave length is obtained by multiplying the value for sodium light by the dispersion factor as obtained above.

BIREFRINGENCE AND DISPERSION OF BIREFRINGENCE OF LENS-CRYSTALS. An elegant way of determining both birefringence and dispersion of birefringence may often be applied to readily fusible organic compounds.

A small plano-convex lens, preferably of fused silica, and an optically worked microscope slide of fused silica are required. The substance to be examined is fused on the silica slide, and the curved side of the lens is brought in contact with liquid, which is then allowed to cool and crystallize. Slight pressure is applied to the top of the lens to ensure contact with the slide. If the operation has been successful, a negative lens of crystal is formed, which has zero thickness at the point of contact of the silica lens and silica slide, and which shows circles of increasing birefringence between crossed nicols.

The appearance with sodium light between crossed nicols of a lens-crystal of α-nitronaphthalene is shown in Figure 6 (left). When crystals of more than one orientation are present, birefringence rings corresponding to the different orientations are produced, as in the case of piperonal (Figure 6, right) where the rings of small diameter correspond to $n_\beta - n_\alpha$, and the larger ones to $n_\gamma - n_\beta$.

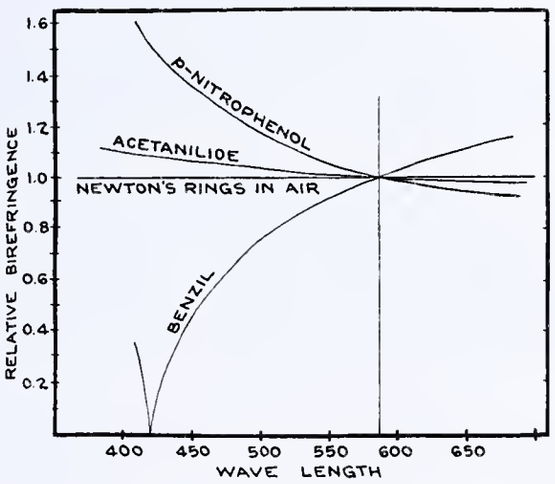

FIGURE 5. TYPICAL CURVES

In actual practice, it is usually necessary to recrystallize the substance by fusion a few times in order to obtain satisfactory lens-crystals. In order to obtain suitable interference figures, it is desirable to have a lens with a relatively large radius of curvature for use with substances of high birefringence, and one of

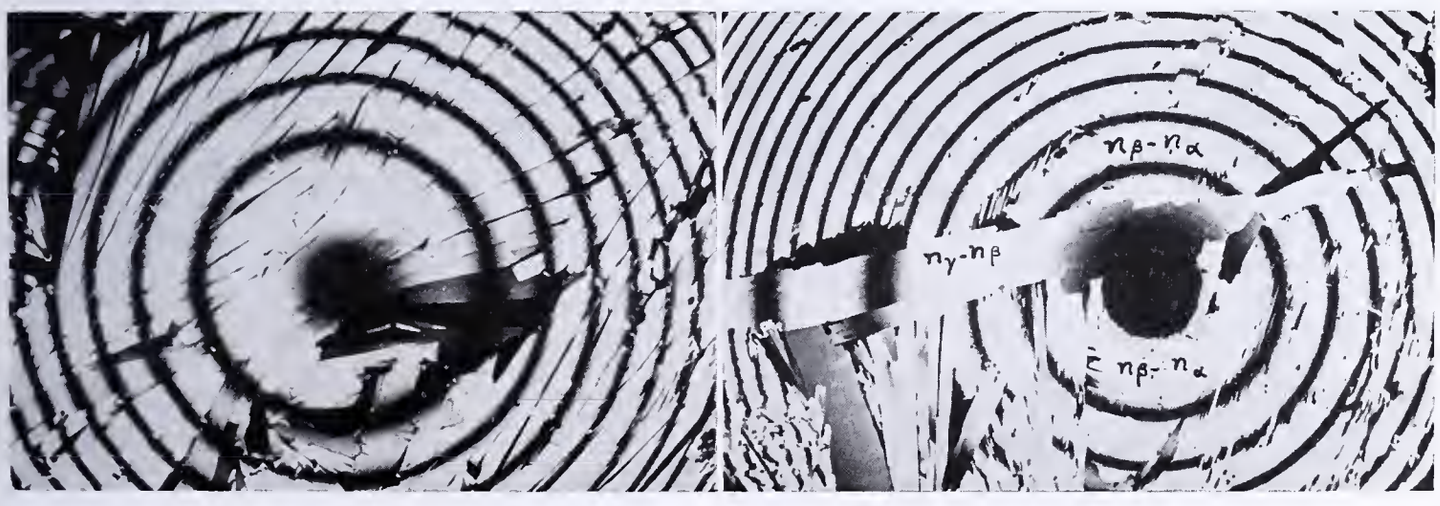

FIGURE 6. BIREFRINGENCE RINGS OF α-NITRONAPHTHALENE AND PIPERONAL

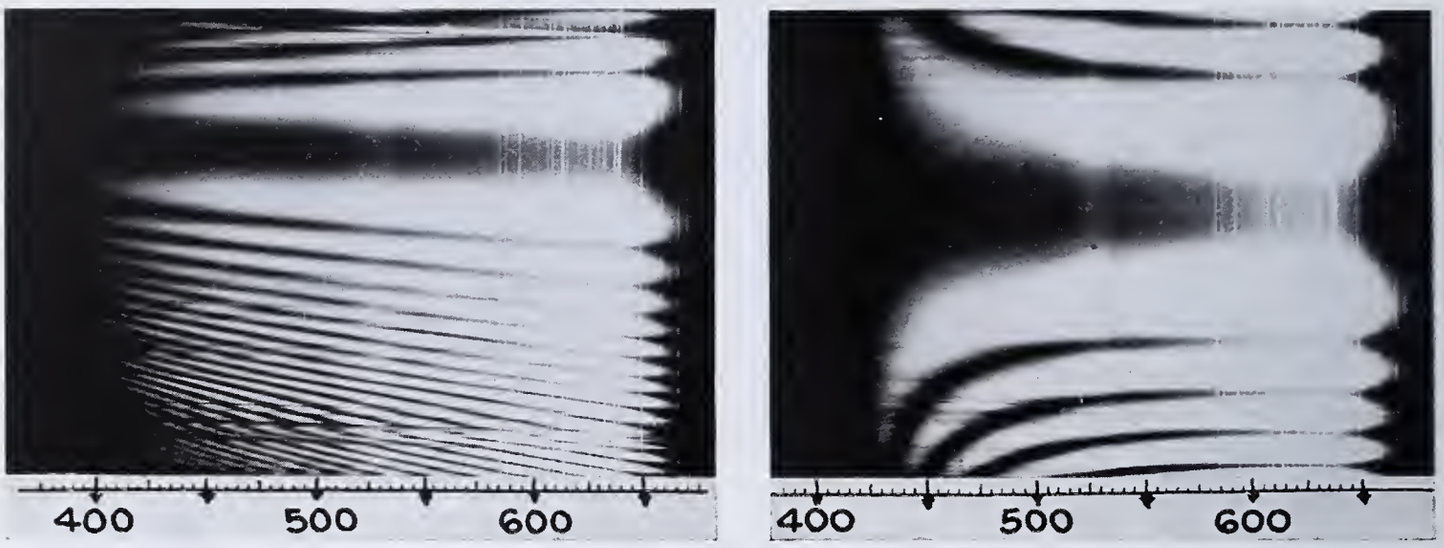

FIGURE 7. BIREFRINGENCE SPECTROGRAMS OF *p*-NITROPHENOL AND BENZIL

smaller radius of curvature for those of smaller birefringence. Those at present in use have radii of 83 and 31 mm., respectively.

Birefringence spectrograms of lens-crystals are obtained at low magnifications, a No. 1 objective and a $\times 5$ eyepiece being used. The diaphragm of the substage condenser is restricted to a numerical aperture of 0.05 in order that the direction of rays of light within the crystal shall not deviate too far from the axis of the lens, particularly when measurements of birefringence are being made for the acute bisectrix. The image of the center of the lens may be thrown to one side of the spectrogram in order to photograph as many interference bands as possible on the other side, but, as it is not easy to mark the exact center of the rings, both first-order interference bands should always be included.

Birefringence spectrograms obtained in this way are shown in Figure 7 (left), which is that of *p*-nitrophenol, and Figure 7 (right), which is that of benzil.

Calibration of the thickness of crystal corresponding to any interference band is best carried out by photographing Newton's rings given in sodium light by the lens in contact with the silica slide. In order to make the rings clearly visible by transmitted light, it is necessary to increase the reflectivity of the lens and slide. The standard way is to half-silver the surfaces, but the author has now found a much simpler method. This consists in applying a drop of a 1 per cent solution of 1,1′-diethyl-ψ-cyanine chloride in methanol to each surface and allowing it to evaporate. The resulting film of dye has an intense narrow absorption band at 578 mμ and, in consequence, a very high refractive index for sodium light. The Newton's rings produced by such treated surfaces are quite as good as those given by half-silvered surfaces, and the dye films are very easily applied and removed, operations which take but a second or so. The Newton's rings are photographed on the same length of 35-mm. Panatomic-X film as is used to record the birefringence spectrograms of the lens-crystals. In order to do this, the slit of the microspectrograph is opened wide, and the grating is rotated to bring either the normally reflected image or the first-order diffracted image to the center of the ground-glass screen. By using the same microscope setup for both the Newton's rings and the lens-crystals, the magnification is unchanged, so that the thickness of crystal necessary to produce any interference band is directly measured in terms of the wave length of sodium light.

A photograph of the Newton's rings is given in Figure 8. As they are produced by transmission, the center is bright, and the air gap corresponding to the nth bright ring has a thickness of $n/2 \times 589.3$ mμ.

Measurements of photographs of both Newton's rings and interference bands produced by the lens-crystals have shown that their diameters vary according to the square root of their order within an accuracy of 1 in 2000, so that it is obvious that the microscopic and spectrographic optical systems are not introducing any appreciable distortion.

In practice, these measurements can be made with adequate accuracy from bromide enlargements. The center of the Newton's rings is found by bisecting the diameter of one of the lower-order rings, and the radius of a high-order bright ring is measured. Let the order of the ring be M, and the radius be X. Measurements are then made from the center of the birefringence spectrogram (obtained by bisecting the distance between the two first-order bands) to the Nth order interference band, for some particular wave length, λ. Let this distance be y. The birefringence of the crystal for this particular wave length is then given by the formula $n_1 - n_2 = 589My^2/2N\lambda x^2$, where n_1 and n_2 are the refractive indices of the crystal for this wave length. Measurements of y are made for various values of λ in order to obtain birefringences for the whole spectrum, and these may be plotted in terms of the value for sodium light in order to obtain dispersion curves corresponding to those obtained by the first method.

FIGURE 8. NEWTON'S RINGS

DISPERSION OF BIREFRINGENCE OF DROPLET CRYSTALS. Fragments of the substance, weighing between 0.1 and 10 mg. are fused on a microscope slide, preferably one o optically worked fused silica. On cooling, the droplets crystallize to yield wedge-shaped crystals. As with the first two methods, it is necessary to check the optical orientation of the crystals by conoscopic observation. In order to determine the dispersion of birefringence, a spectrogram is made of the rising orders of interference of one of the wedge-shaped crystals at a suitable magnification. A droplet crystal of *o*-chloroacetanilide gave the spectrogram shown in Figure 9. The method of computing the birefringence–wave-length graph is identical with that of the first method.

One or other of the above methods will work with a great many fusible organic compounds. Failure to obtain satisfactory crystals may be due to one or more of the following causes:

1. The substance is impure. Two or three recrystallizations from a suitable solvent will often put this right.
2. Conditions of cooling the fused substance are not correct. In general, excessive supercooling should be avoided. A defective wedge- or lens-crystal is cautiously heated so that some of the substance remains unfused and is then slowly cooled. This treatment often yields satisfactory crystals.

Table II. Colors of Platinocyanide Crystals

$(NH_4)_2PtCy_4.H_2O$	Colorless with blue reflection
$(NH_4)_2PtCy_4.2H_2O$	Yellow with blue reflection
$BaPtCy_4.4H_2O$	Green and yellow dichroic
$MgPtCy_4.5H_2O$	Deep yellow
$MgPtCy_4.7H_2O$	Red with blue and green reflections
$K_2PtCy_4.3H_2O$	Yellow
$Y_2(PtCy_4)_3.21H_2O$	Red, yellow, green, and colorless pleochroic with blue and green reflections
Ag_2PtCy_4	Colorless
$HgPtCy_4$	Colorless

3. The fused substance on cooling forms a mass of small crystals of random orientation. Modifying the conditions of cooling should be tried, and if this fails the method for nonfusible substances should be tried.

Dispersion of Birefringence of Nonfusible Crystals. The method of computing the birefringence–wave-length graph described under the first method is readily applied to crystals which possess either a crystallographically or an artificially produced wedge-shaped edge. Such a crystal usually needs to be mounted in some medium having a refractive index reasonably close to that of the crystal in order that the ascending order of interference colors may be seen in the wedge-shaped edge. The mounting medium should be viscous and have as little solvent action on the crystal as possible.

The most generally useful mounting media for crystals of organic compounds are glycerol, glycerol jelly, and "Plasticizer E, 47.7% Cl", supplied by the Hooker Electrochemical Company. The last-named substance has a refractive index, n_D^{25}, of 1.5148. The birefringence spectrogram of the crystal is made to include the wedge-shaped edge, so that the orders of the interference bands given by the thick parallel part of the crystal are readily counted. It often happens that the crystals as grown do not have a wedge-shaped edge and have such strongly defined cleavage planes that they will not give a wedge-shaped fracture. Under such circumstances, a useful procedure is to attack one end of the crystal with a suitable solvent in order to round off the edges. After mounting in glycerol jelly or Plasticizer E, the rounded part of the crystal serves the same purpose as a wedge.

The Color of Crystals

An extensive study of colored crystals has been made by means of the microspectrograph. This work has shown that the spectral absorptions of a crystal depend on both its chemical composition and its crystallographic structure. As a result of this study, it is now proposed to classify the various possible causes of color as follows:

1. Rare-earth elements (Atomic Nos. 57 to 71) give line absorption spectra. Spedding (*7*) has shown that the grouping of lines given by crystals of a rare-earth compound is primarily dependent on the nature of the crystal symmetry. The author (*4*) found that neodymium and praseodymium sulfate octahydrates possess an extraordinary type of pleochroism, characterized by the fact that certain of the absorption bands disappear for specific ray and vibration directions. These directions are different for each absorption band and do not coincide with the axes of the optical ellipsoid.

2. Colored inorganic ions contribute to the color of the crystal. Thus, potassium ferricyanide crystals have a color characteristic of the ferricyanide ion, cupric sulfate pentahydrate crystals have the blue color of hydrated cupric ions, and so on.

When both the anion and cation are colored, their relative contributions may be different for each of the principal absorption spectra, so that the pleochroism of the crystal becomes complicated. In the case of complex cations, it is the arrangement of the coordinating groups, rather than the specific metal atom, which governs the color of the salts. Thus, crystals of the two compounds $[Co(NH_3)_5H_2O]Cl_3$ and $[Cr(NH_3)_5H_2O]Cl_3$ are almost indistinguishable, and both exhibit the same orange and pink dichroism. In the case of rare-earth compounds light is absorbed by electron transitions in the N shell of the rare-earth ion, which is screened by eight electrons of the O shell; hence, the influence of the anion on the absorption is not great.

However, with absorbing anions and cations, the influence of electric fields in the crystal is great, so that the nature of the other ion and of the crystal symmetry may greatly modify the pleochroism of the crystal.

3. The color of the crystal may be characteristic of the molecule as a whole. Under this heading are included inorganic complexes such as $2KNO_2.Cu(NO_2)_2.Pb(NO_2)_2$; metallic coordination compounds such as nickel dimethylglyoxime and the metallic phthalocyanines; organic compounds such as the nitrophenols, nitroanilines, azobenzene, etc. (Iodides, oxides, and sulfides of many metals have a very high absorption over most of the spectrum and may be looked upon as extreme cases of molecular absorption.)

4. Although basic and acidic organic dyes may be considered as colored ions, it is by no means certain that ions exist in crystals of the dye. Organic acids and bases which are colored in solution usually form colored crystals, which means that the absorption-producing mechanism of the dye molecule can contribute to the color of the crystal. The principal absorption spectra of a dye crystal, however, are often unrelated to the absorption spectrum of its solution.

5. Water of crystallization can modify the absorption of a crystal. Sometimes the effect is explainable on the assumption that the water coordinates with a colored ion to yield a complex ion having a different spectral absorption. This, for example, is the case with cobalt chloride; crystals of $CoCl_2$ are blue, and those of $CoCl_2.6H_2O$ are red, the hexahydrate formula probably being more accurately represented by $[Co(H_2O)_6]Cl_2$. This theory receives support from the fact that a saturated solution of cobalt chloride in water is red at room temperature, but becomes blue when the temperature is raised. Furthermore, solutions of $CoCl_2$ in anhydrous organic solvents are blue.

Such an explanation will not account for the extraordinary colors of crystals of platinocyanides which are colorless in solution. That the presence of water of crystallization is responsible in some way for the production of a light-absorbing mechanism is obvious from a study of Table II.

It will be noted that the two anhydrous platinocyanides, those of silver and mercury, are colorless. The ammonium salt with one molecule of water has a strong absorption in the near ultraviolet and has, in consequence, an abnormally high refractive index in the violet which, in turn, is responsible for the selective surface reflection of the extreme blue end of the spectrum. It appears to be a general rule that the greater the molecular proportion of water in a platinocyanide crystal, the lower the vibration frequency of the absorbed light, and it is interesting to note that both the red magnesium and yttrium salts have seven molecules of water to each platinocyanide ion.

The effect of water of crystallization in crystals of basic dyes is usually great, the hydrated crystal being different from the anhydrous crystal in crystal form, birefringence, and principal absorptions. Curiously enough, the color of the nonhydrated crystals often bears most resemblance to the color of the solution, which is an indication that water of crystallization in basic dyes may become part of a new absorption mechanism.

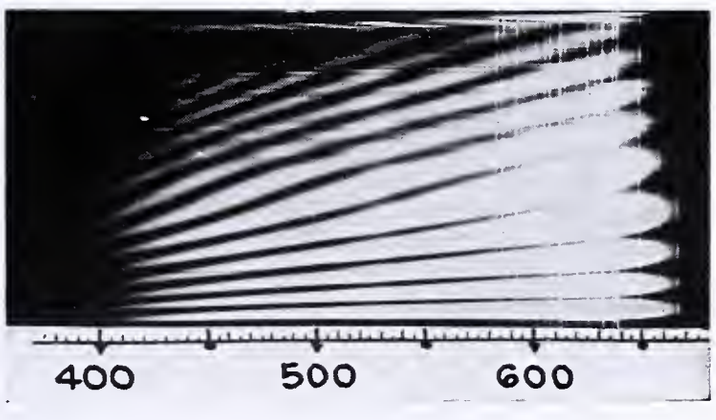

Figure 9. Spectrogram of *o*-Chloroacetanilide

6. The degree and nature of the optical anisotropy of a crystal influence both the intensity and position of the absorption bands. The author has studied anhydrous crystals of 26 different salts of the basic dye 1,1′-diethyl-ψ-cyanine and has found that those of high birefringence have strong absorption and pleochroism, whereas those of low birefringence have not only weak pleochroism but also a very weak depth of color. Crystals of the iodide, 20μ thick, are very deep red in color, whereas a similar

thickness of the trithionate is only very faintly yellowish orange. The dithionate and tetrathionate are rather more birefringent and have slightly more color. The *p*-toluenesulfonate and *β*-naphthalenesulfonate come next in the list and are orange in color. All these crystals give the same magenta-colored solution in methanol. Solvent of crystallization (other than water, which has specific properties discussed above) may increase or decrease the anisotropy and depth of color of the crystal. Thus in the study of the 1,1′-diethyl-*ψ*-cyanine salts it was found that, whereas crystals of anhydrous chloride had nearly the same depth of color as the iodide, crystals of chloride containing phenol of crystallization had a much lower birefringence and were relatively weak brownish orange in color. It appears to be a general rule that a decrease in anisotropy is associated with a shift of the absorption band towards the shorter wave lengths.

These six factors often operate in combination, particularly with crystals of salts in which both anion and cation are dyes. For example, the chrysophenine salt of 1,1′-diethyl-*ψ*-cyanine forms yellowish-orange crystals having a relatively weak birefringence. The predominant color is, therefore, that of chrysophenine. A similar effect has been observed with crystals of picrates of basic dyes; the strong yellow of the picrate ion is not much affected by the cation, whereas the light-absorption of the dye cation may be greatly changed in wave length and intensity.

It is evident that considerable care must be exercised in examining unknown dyes and comparing their optical characteristics with those of known dyes, but the above considerations do at least indicate some general rules. Thus both known and unknown should be converted to the same salt, and both should be recrystallized three or four times from the same solvent. So far as basic dyes are concerned, the perchlorates are the most satisfactory salts on account of their low solubility, lack of tendency to take up solvent of crystallization, and freedom from color of the perchlorate ion. As an example, methylene blue perchlorate is precipitated when an excess of sodium perchlorate is added to an aqueous solution of methylene blue chloride. The precipitate is washed and then recrystallized from methanol. Very few technical acid dyes can be induced to form crystals suitable for microscopic examination, but some degree of purification can usually be obtained by precipitation as the free acid and re-solution in ammonia, repeating the cycle several times.

Crystals of dyes must be extremely thin for microscopic examination. The appearance of a colored crystal in ordinary light, with one nicol, and with crossed nicols, may vary very considerably with change of thickness, so it is necessary to study a range of thicknesses. Evaporation of thin films of very concentrated dye solution is one of the easiest ways of preparing crystals for study, suitable solvents for this method being pyridine and benzyl alcohol.

α-Bromonaphthalene and *α*-chloronaphthalene have useful solvent properties. At room temperature they are poor solvents, but at temperatures of 100° to 300° C. they become powerful ones. Consequently, it is possible to perform recrystallizations on microscope slide preparations by heating some finely ground dye under a cover slip with *α*-bromonaphthalene or *α*-chloronaphthalene and then allowing the preparation to cool very slowly. The high index of refraction of these solvents is of particular advantage, as most dyes have very high values of n_β and n_γ.

It has been found that there is only one certain way of studying the pleochroism of strongly absorbing crystals. This consists in confining the area of illumination to within the boundaries of the crystal. If the illuminated area is greater than the size of the crystal, the color is degraded in two ways; intersurface reflections in objective and eyepiece dilute the color of the crystal with white light, and light reflected backwards by the objective is reflected by the surface of the crystal to such an extent that the surface color is often the only one seen in the microscope. A striking example of this effect is shown by crystals of 1,1′-diethyl-*ψ*-cyanine perchlorate. Examined in the ordinary way, with the microscope slide illuminated with polarized light filling the entire field of view of the microscope, the crystals appear to be colorless and pale brown dichroic, but when the illumination is restricted to the crystal, the dichroism is colorless and very deep crimson.

The swing-out type of condenser, which is usually fitted to petrographic microscopes, is not well adapted for work on very small colored crystals, as it is difficult to illuminate a small enough area of the slide. The author now uses either an achromatic condenser or a 4-mm. achromatic objective (N. A. = 0.65) as a condenser, by means of which the image of a distant tungsten arc lamp or ribbon-filament lamp is focused on the crystal under observation. Double reflections from the microscope mirror have been overcome by having it aluminized. For visual work the dichroism of the crystal is studied by inserting the analyzer in the path of light, no polarizing prism being used. Neither the polarizer nor the analyzer is used for microspectrographic work; instead, the image of the crystal or its conoscopic image is projected on the slit of the microspectrograph, and the Wollaston prism is used to produce two adjacent spectra corresponding to the vibration planes of the crystal. In order to set the crystal so that its vibration planes agree with the vibration planes of the Wollaston prism, the microscope polarizer is temporarily inserted and rotated until it extinguishes one of the images given by the Wollaston prism, and the crystal is set at extinction. The polarizer is then withdrawn and the spectra are photographed.

It was mentioned above that strongly absorbing monoclinic and triclinic crystals may have strong dispersion of the axes of the optical ellipsoid. When this is the case it becomes impossible to make dichroism spectrograms which represent principal absorption spectra. The use of a sodium vapor lamp for the preliminary study of interference figures of colored crystals is strongly recommended, as this greatly simplifies the task of interpreting the usually highly complicated interference figures obtained with white light. Sodium illumination is also of considerable service in studying figures presented by the obtuse bisectrix and normal to the optic axial plane, as even thick crystals give clearly defined "hyperbolic" figures under these conditions.

Surface Color. Colored crystals which possess a strong absorption in some region of the spectrum often have a strong surface color in consequence. If the strong absorption is confined to one vibration direction of the crystal, the selectively reflected light is polarized, and consequently, trichroic crystals may reflect different colored light from different faces and exhibit a "reflection dichroism". The refractive index of an absorbing crystal is abnormally high on the red side of the absorption band and abnormally low on the blue side. In dichroic crystals these abnormalities in refractive index occur only for the ray which is absorbed, and the other ray does not show them. Consequently, the sign of the birefringence may be reversed twice in the visible spectrum. The color of the surface reflection is on the red side of the absorption band for crystals mounted in air, but on the blue side for crystals mounted in a medium of high refractive index, such as *α*-iodonaphthalene; consequently, it is necessary to mount crystals for comparison in the same mounting medium. The surface colors of many basic cyanine dyes have been examined with the microspectrograph. Well-formed faces of the crystals were illuminated with a cover-glass illuminator between the objective and crystal. Reflected light from the crystal was analyzed with the Wollaston prism. No sharp maxima or minima were observed.

The surface reflection of very small crystals is best studied by means of a prism vertical illuminator equipped with a 2-

mm. oil-immersion fluorite objective in a short (metallographic) mount. The objective must be specially selected for freedom from birefringence. An analyzer is used in the microscope for visual examination, and the stage is rotated in order to observe reflection dichroism. For microspectrographic work, the Wollaston prism serves as an analyzer. The vertical illuminator is used without a polarizer for this work. This particular aspect of the optics of strongly absorbing crystals is worthy of further study by the chemical microscopist, as the surface color of such crystals is independent of their thickness.

Acknowledgments

In conclusion the author wishes to thank H. D. Babcock for the gift of a speculum grating, J. L. Houghton and Max Wiedling for valuable assistance in the construction of the microspectrograph, Fred Lee for the gift of a specially worked sapphire disk, and L. G. S. Brooker and Frances M. Hamer for many splendid specimens of strongly absorbing crystals.

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PRESENTED before the Division of Microchemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich. Communication 781 from the Kodak Research Laboratories.

An Electric Heating Mortar

For Use in Carbon and Hydrogen Microcombustions

G. FREDERICK SMITH AND WM. H. TAYLOR
University of Illinois, Urbana, Ill.

THE heating mortar usually employed in carbon and hydrogen microcombustion analyses has not proved entirely satisfactory, as this glass heating device containing boiling cymene with an air-cooled reflux condenser and gas microburner is both fragile and cumbersome. Another disadvantage is insufficient variability of temperature adjustment. This type of heating mortar has been improved upon by Schneider and Van Mater (*2*), who use electrical heating with thermostatic control. A second improved electrically heated and thermostatically controlled heating mortar, known as the "micro thermostatic sleeve", is now commercially available (*1*).

The present discussion has for its object the description of an electric heating mortar, thermostatically controlled, which is compact, simple in construction and operation, and at the same time provides constant temperature control over a comparatively wide range of temperatures. For the most part standard units of laboratory equipment are used. The construction of the remaining parts involves simple machine tool manipulations.

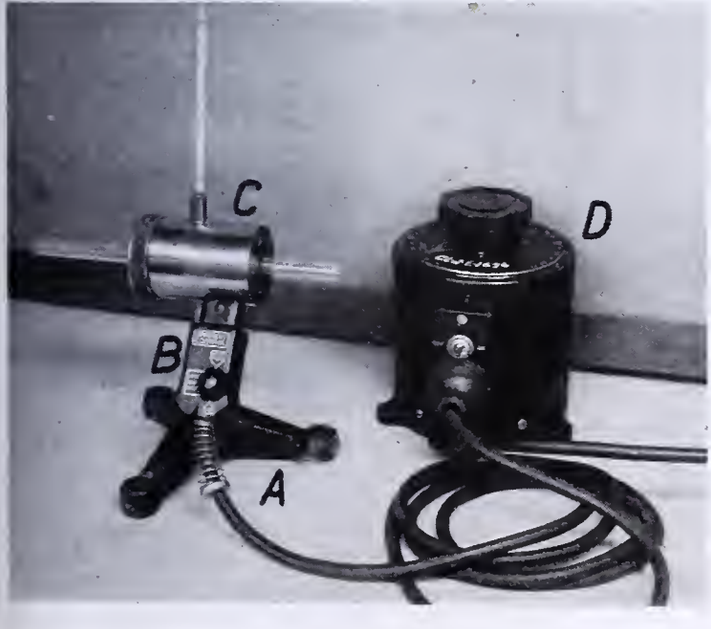

FIGURE 1. HEATING MORTAR

In Figure 1 the heating mortar with thermometer well, thermometer, and a section of standard combustion tubing is shown at *C*. The electrical thermostatic control switch ("Anyheat control", Silex Corporation, Hartford, Conn.) is shown at *B*. The voltage control (Variac Type 200-C, General Radio Corporation, Cambridge, Mass.), shown at *D*, may be replaced by a suitable lamp-bank voltage control. The adjustable support for the heating mortar is mounted on the tripod base, *A*. By its use the heating chamber may be raised or lowered.

The quarter-sectioned diagram (Figure 2) shows details of the construction of the heating mortar. The shaded portion with alternate solid and dotted lines indicates brass. The criss-cross shaded areas indicate Transite. The close-spaced diagonal lines indicate graphite. The circles with diagonals indicate Nichrome.

The barrel of the heating mortar is 8 cm. long with the screw cap shown at the left fully extended and is 48 mm. in outside diameter. The holes at each end and through the graphite disks are 11 mm. in diameter. The electrical contacts for attachment of the thermostatic control switch are 4.5 mm. in diameter and extend approximately 15 mm. from the body of the heating mortar, with a 1-cm. space between.

The casing of the heating mortar serves as one electrical contact, and the brass shoulder at the left of the Transite disk insulating ring at the exit end serves as the other electric contact. The side walls of the barrel of the heating mortar are insulated by use of a Transite tube as shown. The electrical circuit is thus through the casing (using the right-hand lug which is insulated from the casing) into the resistors and out through the right-hand "Anyheat control" lug.

(According to the assembly as described, if the electrical contacts of *B* are not checked a short circuit may result between the operator and a ground connection. This point should be checked, using a voltmeter, and reversing the contacts either at the power supply line or the thermostatic control switch from the transformer, so that no short circuit will be possible.)

The carbon disks (Figure 2) are 37.5 mm. in diameter and 1.5 mm. thick (Allen-Bradley Co., Milwaukee, Wis., Type E-2910 rheostat graphite disks). A sufficient number are slotted as shown, to provide for an opening below the thermometer tube. The Nichrome resistors are 37.5 mm. in diameter and are made from 3-mm. wire cut from a suitable wound helix.

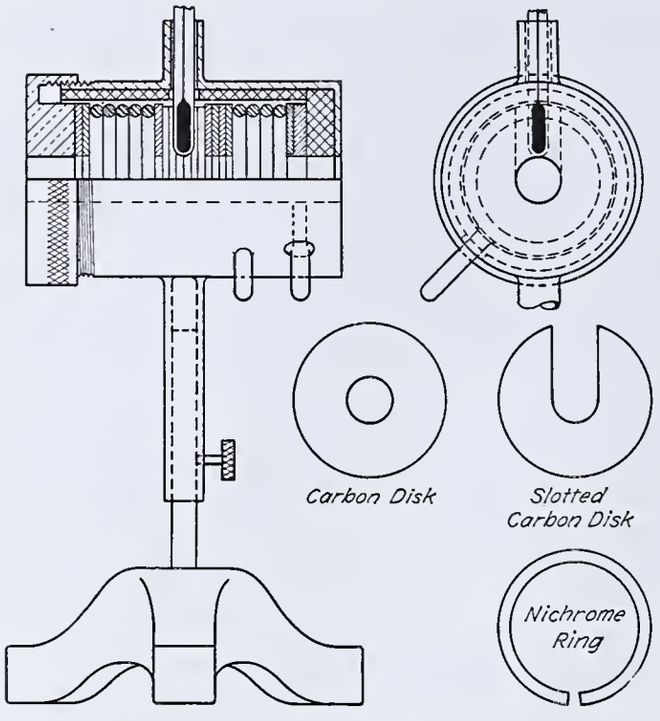

FIGURE 2. DETAILS OF CONSTRUCTION

The thermometer tube is 18 mm. long, 1 cm. in outside diameter, and of 6-mm. bore. The tripod upright is 55 mm. long, and of 6-mm. diameter to provide a slip joint with 55 mm. of the same tubing as that used for the insertion of the thermometer. The heating mortar is supported in contact with it by means of a setscrew as shown, for adjustments in height. All soldered connections are made with silver.

The carbon disk resistors in the middle portion of the mortar provide for the production of less heat energy than that from the Nichrome rings at either end, which brings about an equal heat distribution not attainable if all heating elements are either graphite or Nichrome. The resistance of the whole mortar is diminished or increased in proportion to the tension applied by adjustment of the screw cap. The "make" and "break" of *B* may be adjusted by altering the position of the adjusting knob to higher or lower temperatures. The Variac control is powered by the regular 110-volt laboratory power source. It is used over the voltage range 5 to 15 volts and supplies the third heat control.

The thermometer is an Anschütz type having a range of 150° to 220° C. Temperatures at the thermometer well in the middle of the heating mortar can be readily adjusted at any point between 150° and 220° C. and maintained constant within 1° to 2°. The variation from center to extreme ends can be kept below 5° temperature gradient.

The heating mortar described has been used for 6 months in the authors' microchemical laboratory at the University of Illinois with perfectly satisfactory results and its life span should be practically endless, without need for further adjustments. The temperature at which it is operated does not alter the carbon-to-Nichrome resistance contact in a measurable degree. The heating mortar has been operated continuously for 30 days with no change required in its three temperature-adjusting variables; during this period there was no change in temperature that could not be attributed to changes in room temperature.

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Volumetric Flasks for Microanalysis

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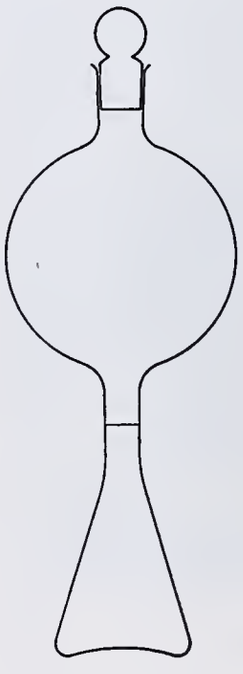
WHEN solutions are mixed in small volumetric flasks of the usual design, the error caused by the trapping of pure solvent or partly mixed solution in the ground glass at the stoppered end of the flask is proportionately much greater than that caused by the same source of error in large flasks.

Moreover, thorough mixing of solutions in conventional flasks of 5- or 10-ml. capacity is difficult to accomplish rapidly. Both this source of error and this lack of convenience in mixing are avoided by the use of flasks of the design shown in the figure.

In such a flask the upper part has a capacity about five times that of the calibrated part, so that after the solution is made up to the mark it may be passed into the upper part by tipping the flask on its side. Then by righting the flask and at the same time giving it a slight circular motion the liquid is caused to swirl as it passes back into the lower part. By repeating this about three times the solution is thoroughly mixed.

The sides of the calibrated lower part should slope more than in flasks of the usual design, in order that the liquid may be entirely, or almost entirely, transferred to the upper part without coming into contact with the upper neck. The neck of the calibrated part may be made slightly smaller in diameter than the neck of conventional flasks, with a corresponding gain in accuracy of measurement. For use with aqueous solutions and micropipets of the usual outside diameter, the internal diameter of the neck cannot, however, be less than about 8 mm. When these flasks are to be used with aqueous solutions there is no need for a ground-glass stopper, but with solutions containing volatile solvents this is, of course, almost necessary.



Though these flasks are considerably taller than conventional flasks of corresponding capacity, they may be completely emptied by micropipets of the bulbless capillary type. The chief disadvantage is the tendency to tip over easily. This disadvantage is of no significance if such a flask is habitually placed while in use in a suitable support such as a small iron ring or the opening in a wooden funnel rack.

Small flasks of this design may be blown without much difficulty from glass tubing.

MODERN LABORATORIES

New Science Building at Kansas State College

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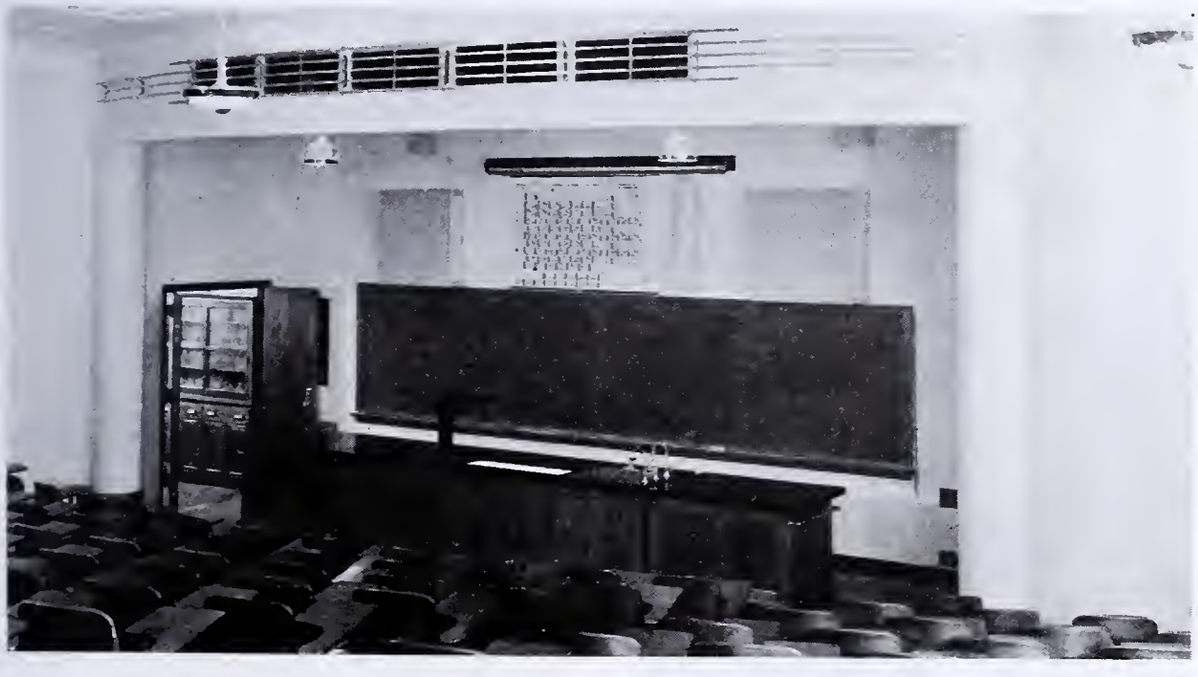
THE Chemistry Department of the Kansas State College of Agriculture and Applied Science is enjoying the use of new Science Building, after having occupied very cramped quarters since the old building burned in July, 1934. The new building has been named Willard Hall in honor of J. T. Willard, who served as head of the department for many years.

Willard Hall is built of native limestone and trimmed with Bedford limestone. The building is 305 feet long by 65 feet wide with wings measuring 92 by 66 feet placed at each end, giving it the shape of a large E with the center bar missing. The interior is of fireproof steel and reinforced concrete construction; only the doors and furniture are of wood. The floors are of concrete except for the classrooms and offices in which a wood floor has been laid on top of the concrete base. A WPA grant was obtained to aid in the construction and equipment of the building, the total cost of which was about $725,000.

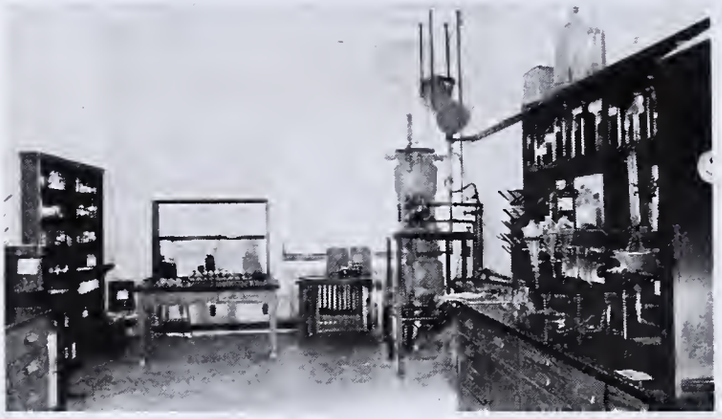
The four floors were carefully planned to make maximum use of the floor space available. An 11-foot corridor runs lengthwise of each floor, dividing it into a 25-foot section toward the front of the building which is used for student work and an 18-foot section toward the rear, in which are placed the remainder of the student laboratories, the service rooms, and the offices and research laboratories of the teaching staff. Stairways are located at each end of the building and in the center rear. Short connecting corridors lead from the center corridor of the main or second floor to the main entrance at the center of the building and to flanking front entrances located toward each end. Other entrances at ground level are located at each end of the building and opposite the main entrance at the rear. The intersection of corridors at the main entrance has been made into a small rotunda, on the floor of which is a mosaic made up by working brass inlays of some of the more important alchemical symbols into a terrazzo background. Large glass-front display cases are recessed into each wall of the short corridor leading to the main entrance.

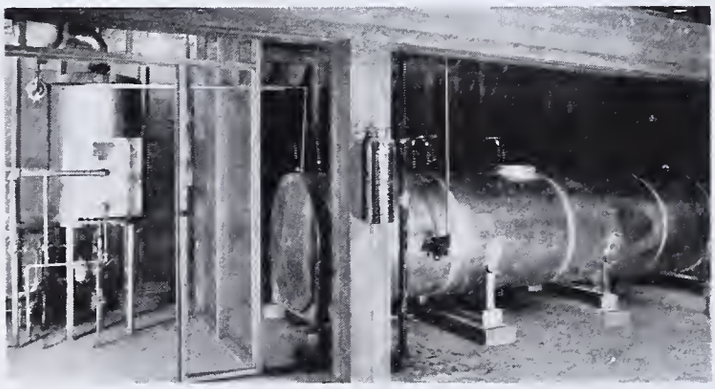
The Department of Chemistry occupies about two thirds of all four floors, while the remainder of the building is given over to the Physics Department.



CHEMISTRY LECTURE ROOM, SHOWING LIGHTING SYSTEM AND GRILL FOR VENTILATING SYSTEM OVER LECTURE TABLE

Preparation room is shown through door on left. Right end of lecture table has been detached; left end is of same construction, both ends may be rolled into preparation rooms. White strip on table is illuminated panel. Loud speakers for sound motion pictures are on both sides of periodic chart.





Chemistry Department

First Floor. The first floor houses the laboratories of the Agricultural Experiment Station. In this is included research on starch and other chemurgic problems, soils, animal nutrition, meat, dairy, and poultry, each occupying separate rooms designed and equipped to meet special needs. A suite of two large laboratories, weighing room, and sample grinding and storage room is set aside for the analytical laboratories of the Experiment Station. Here also is done analytical work for the State Board of Agriculture. In another laboratory the analytical work for the State Board of Health is done. A special laboratory which has no gas connections—all heating is done with steam or electricity—is available for work with and the recovery of inflammable solvents. A small animal room; a well-equipped shop with facilities for glass, metal, or wood working; the electrical control panel with its accompanying bank of storage batteries and motor-generator equipment; the compressor and vacuum pumps; the receiving room; and a washroom with shower facilities are all on this floor.

One of the most interesting features is a battery of five constant-temperature and constant-humidity rooms. Each room is separately controlled and the temperature range available is from $-30°$ to $50°$ C. Any relative humidity between 10 per cent and saturation can be maintained. Another constant-temperature room is connected to the laboratory for soil research. Four laboratories housing special research equipment for physical chemistry, and the Experiment Station Office, with a fireproof vault for the storage of records, complete the facilities on this floor.

Included in the special equipment are several large centrifuges, a supercentrifuge, high-pressure hydrogenation equipment, two large Carver presses, a Kjeldahl digestion rack of 60-flask capacity and a distillation rack capable of handling 36 flasks, a vacuum oven, a large thermostat, a liquid air machine, complete x-ray equipment, a large spectrograph with both glass and quartz prisms and capable of giving a 30-inch spectrum, complete equipment for spectrographic and spectrophotometric work, a photoelectric colorimeter, and a fluorophotometer for absorption in the ultraviolet region. An office desk, desk chair, and filing case have been placed in each research laboratory.

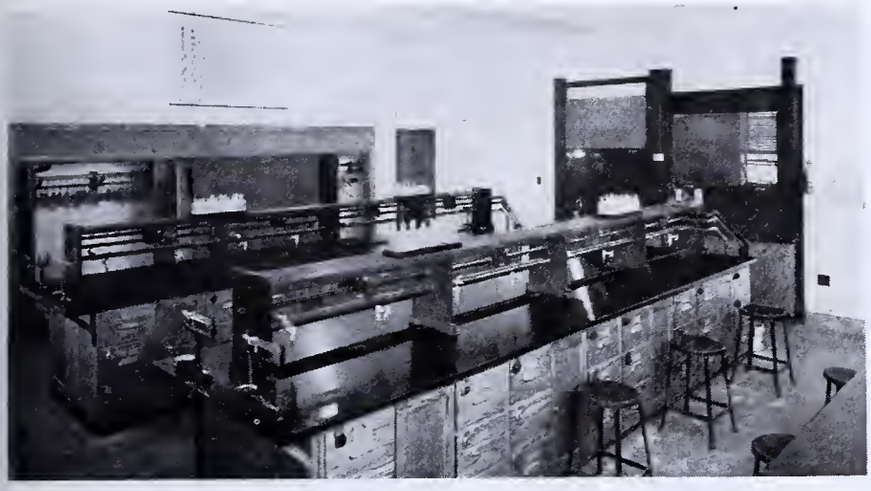
Second Floor. The department offices, located on the second or main floor, consist of a suite of four rooms made up of the business office, with another fireproof vault for records; the general office; and a private office and research laboratory for the head of the department. A two-way buzzer system connects the general office with all the offices for the staff and with the laboratories, both student and research. Communication is further aided by a telephone (intercommunicating and outside) located on each floor. The department library is across the hall from the general offices.

Top. Typical Office–Research Laboratory for Teaching Staff. Left Half of Room Is Similar to Section Shown in Photograph

Second from Top. Laboratory of the Agricultural Experiment Station, Used as an Extraction Room for Work with Volatile Solvents

Third from Top. Water Still and 1000-Gallon Pure Aluminum Storage Tank in Attic. By Using Pure Aluminum in Tank and Distributing Pipes, with Aluminum and Silver Faucets, a Plentiful Supply of Distilled Water with a Conductivity of 10^{-6} Reciprocal Ohm Is Available throughout the Building

Lower. Typical Desk Arrangement in Quantitative Laboratory, Showing Balance Room at Right



TYPICAL FRESHMAN LABORATORY, SHOWING COMBINATION BLACKBOARD AND CLOTHES LOCKER. EACH STUDENT IS ASSIGNED A DRAWER. CUPBOARD HOLDS EQUIPMENT USED IN COMMON BY SIX SECTIONS

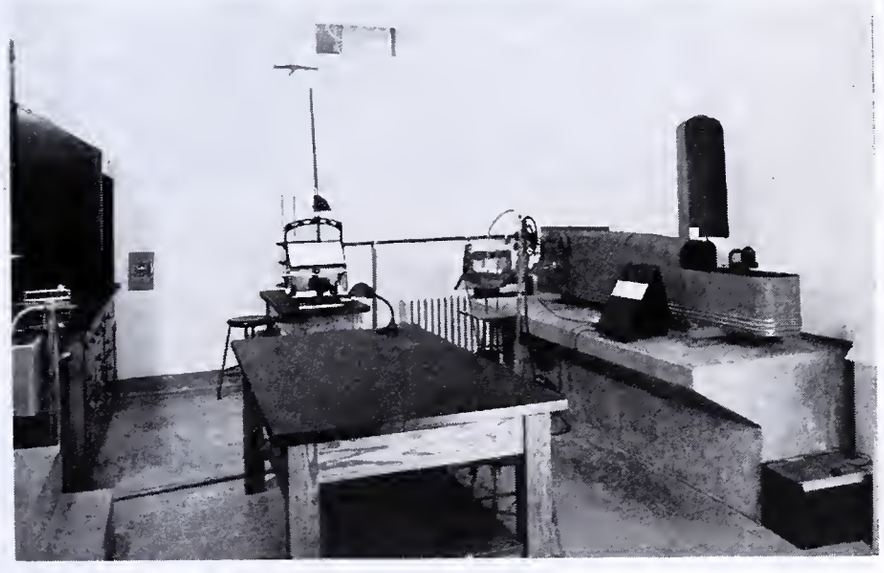
classroom seating 40, and two offices and research laboratories for staff members.

THIRD FLOOR. On the third floor are laboratories for organic chemistry. Two laboratories, used by the elementary courses, will accommodate 416 students working in four sections. A third laboratory, for students majoring in chemistry, can be used by three sections or a total of 111 students. The physiological chemistry laboratory will also accommodate 111 students in three sections. A small laboratory with desks for eight students may be used for organic analysis and organic preparations.

The physical chemistry laboratory will accommodate two sections of 24 students each. Adjoining it are a special supply room and a smaller laboratory in which the more elaborate pieces of apparatus may be set up.

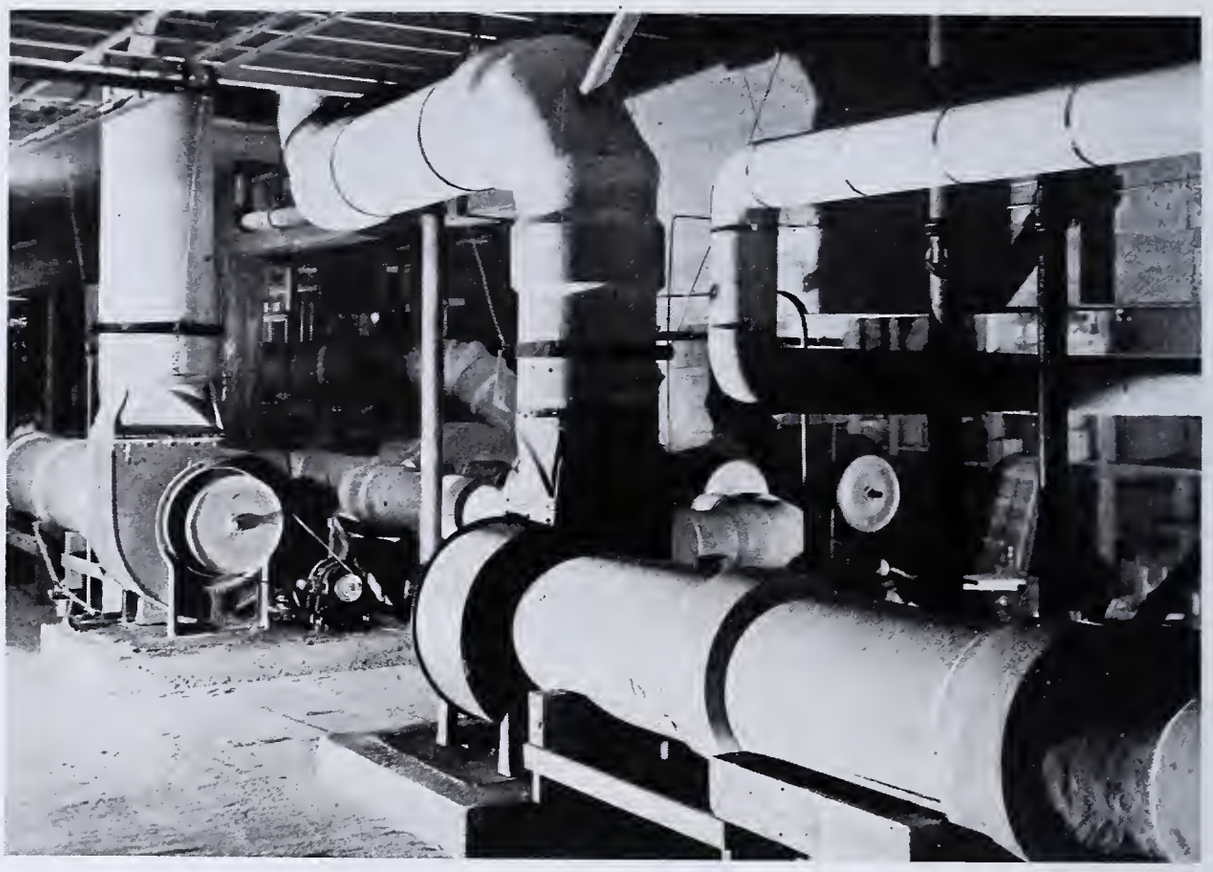
The lecture room occupies the entire wing, with 233 seats arranged in amphitheater fashion. It has indirect lighting and a separate ventilating system. A long, thoroughly modern lecture table occupies the front of this room. The end sections of this table may be detached and rolled into the preparation rooms at each side. These lecture preparation rooms are completely equipped and each may be reached by a spiral stair from the first floor. The lecture table is equipped with hot, cold, and distilled water, gas, air, vacuum, 220-volt alternating current, and variable direct current. The large sink may also be used as a pneumatic trough, and a display plate, made by mounting a ground-glass plate in the top of the table and illuminating it from below, is an aid in making the details of lecture demonstrations plainly visible. A small but powerful table hood removes obnoxious gases. The lighting system of the room, as well as the mechanical shades covering the windows, may be controlled from the lecture table. A 70-slide automatic Baloptican (also controlled from the lecture table) and a motion picture machine for both sound and silent pictures are available for visual education. The sound equipment is so arranged that the lecturer may, if he chooses, operate either the motion picture machine or the projector from the back of the room; yet, by speaking into a small microphone, his voice will reach his hearers from the front of the room.

The facilities for quantitative analysis include a laboratory capable of accommodating two sections of 40 students each. Attached to this laboratory but separated from it by glass partitions are a balance room and a sample preparation room. The advanced inorganic preparations laboratory may be used by two sections of 24 students each, while an electrochemical laboratory with 16 desks and a graduate research laboratory with 32 desks complete the arrangement for student laboratory work on this floor. Here also are a



TOP. ONE OF THE RESEARCH LABORATORIES FOR PHYSICAL CHEMISTRY, SHOWING SPECTROGRAPH PLACED ON CONCRETE PIER

BOTTOM. ONE OF THE ORGANIC LABORATORIES. BECAUSE THIS LABORATORY IS LOCATED OVER THE LECTURE ROOM, THE SERVICES ARE DELIVERED TO DESKS FROM CEILING MAINS. IN OTHER LABORATORIES, THE SERVICES COME FROM BELOW



VENTILATING SYSTEM IN ATTIC

In the background, behind network of pipes, is one of the cages for storage of chemicals and equipment.

A classroom seating 40 persons completes the facilities for student work on this floor. Three combination offices and research laboratories and two offices with research laboratories attached are assigned to the nine staff members who work principally with organic, physiological, or physical chemistry.

FOURTH FLOOR. On the fourth floor are five inorganic chemistry laboratories which will accommodate a total of 1308 students working in six sections. Three combination offices and research laboratories and one office with research laboratory attached are used by the nine staff members whose work is with the freshman chemistry.

The laboratory desks are built of oak and were designed to meet the requirements of the classes or individual using them. The desk tops are of a new cement-asbestos composition and are almost impervious to ordinary laboratory reagents. An open drain lined with heavy lead sheeting runs through the middle of each table and empties into a Knight-ware sink at the end. Distilled water is delivered through aluminum pipes to a faucet at the end of each laboratory table. A water-steam mixing bulb is placed over each sink, so that either hot or cold water is always available. Each working place is provided with outlets for water, gas, compressed air, and vacuum. In the organic and physiological laboratories a steam cone is placed at each working place. The laboratories used by upperclassmen have available also a 220-volt alternating current outlet and a variable direct current outlet. Reagent shelves are placed conveniently along the walls of the laboratories. In the freshman laboratories two triple-beam balances with 111-gram capacity are placed at the end of each table opposite the sink. In the other laboratories a triple-beam balance with 1100-gram capacity is mounted on each reagent shelf. Each student laboratory has a large clothes locker occupying one whole end of the room. Blackboards are mounted on the doors of this locker space.

The variable direct current originates with a bank of storage batteries on the first floor, and the necessary motor-generator set is available to keep these batteries charged. From a master control panel board the current selected, which may be anything from 2 to 110 volts, is distributed to subpanel boards located on each floor and from there to the individual laboratories. In the larger student laboratories there are at least two circuits available, so that two widely different experiments may be in progress at the same time.

The laboratory hoods are arranged in vertical banks throughout the four floors, each with its own motor and exhaust fan in the attic. These fans continue to function as long as any hood of a bank is turned on. The individual units are controlled by a system of dampers operated by the motor switch; hence, the exhaust fan is effective only on those hoods which are turned on. In the large student laboratories are placed Univent ventilators which operate simultaneously with the hood fans and in this manner compensate for the air removed through the hoods. Each hood is provided with gas cocks, an electrical power outlet, water, and drain. The larger hoods have electric lights installed toward the top of the hood.

The main storeroom for the department is in the attic. All incoming supplies are unloaded into a receiving room on the first floor, where they are unpacked and entered on stock records. The distribution may be to the service rooms, one on each floor, or to the attic for storage. A freight elevator connects this system of storerooms. The attic also houses a 50-gallon-per-hour Barnstead water still and a 1000-gallon aluminum storage tank for distilled water. The hydrogen sulfide for the qualitative analysis laboratories is bought in commercial cylinders. The pressure is regulated by an oil-filled gasometer, placed in the attic, and from there the dry gas passes through cast-iron pipes to jets located in the laboratory hoods. All volatile and inflammable solvents are stored separately in an underground vault which is reached directly from the receiving room.

INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY • HARRISON E. HOWE, EDITOR

Papers from the Symposium on Vitamins presented before the joint meeting of the Divisions of Biological and Agricultural and Food Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich., pages 209–31.

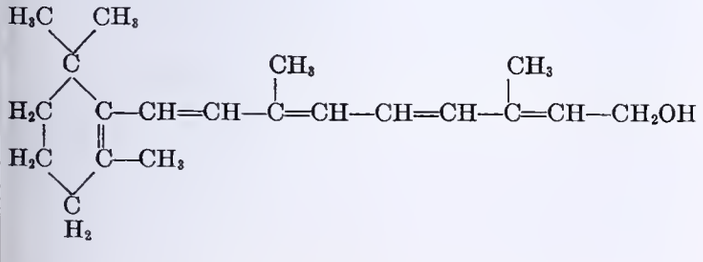
Physical and Chemical Determination of Vitamin A

J. B. WILKIE

U. S. Food and Drug Administration, Washington, D. C.

THE existence of vitamin A was biologically established about 25 years ago, and since that time its importance for health and nutrition has become generally recognized. Coincidentally three general methods for its assay have received much attention. One of these, the biological method, is well established and is now recognized by the U. S. Pharmacopoeia as official. However, because of the time involved and the expense associated with the biological method, much effort has been expended upon possible physical and chemical procedures for this vitamin. The literature upon the subject is relatively voluminous and contains many contradictions, but enough work has now been completed to indicate either the causes or the remedies for many of the earlier difficulties. It is a principal purpose of this review to indicate the current status of the physical and chemical determinations of vitamin A.

The accumulated knowledge of the vitamin A determination is largely empirical and not until recently has its structure been determined. A study of this structure may help to determine causes of error in the physical and chemical estimation of this substance. We see by reference to the structural formula that it consists of a beta-ionone ring attached to an unsaturated chain with methyl groups and ending with an OH group.



Antimony Trichloride Reaction

The earlier efforts toward the estimation of vitamin A dealt largely with chemical reactions. The observation was made that cod liver oils would give color with various types of reagents such as arsenic trichloride (*39*), sulfuric acid (*46*), and antimony trichloride (*49*). As early as 1880 a chemical test for the identity of cod liver oil in the U. S. Pharmacopoeia was based upon the action of the oil with sulfuric acid. Thus it appears that a test for vitamin A was available before the vitamin was discovered biologically.

Carr and Price (*4*) in 1926 studied the analytical possibilities of such a reaction more thoroughly than had been done previously. They established the use of antimony trichloride in chloroform as a quantitative reagent but found that the color resulting from its reaction with vitamin A faded rapidly. When this color was examined spectrophotometrically the 620 and 583 mμ bands were found to predominate in concentrates while 606 and 572 mμ bands were found in unconcentrated oils. The antimony trichloride test has been published as a recommended method for concentrates in the British Pharmacopoeia.

A 20 per cent solution of the oil is prepared at a specified temperature (20°); 0.2 cc. of this solution is dropped into a 1-cm. cell and the oil is placed in a colorimeter or a tintometer. Then 0.2 cc. of the reagent (antimony trichloride) in dry chloroform is added in such a manner as to mix rapidly.

Many other investigators (*2*, *3*, *7*, *17*, *18*, *21*, *30*, *32*, *33*, *34*, *36*, *37*, *43*, *45*) have shown that the antimony trichloride reaction is normally susceptible to various factors affecting the developed spectral bands. Changes in these bands may be due to interfering materials, such as inhibitors, or to lack of proper precautions in technique, particularly control of temperature, improper preparation of reagent, or lack of speed in making readings.

For certain classes of materials the numerous investigations indicate that the antimony trichloride reaction with the proper precautions will give results of quantitative significance having about the same accuracy as the ultraviolet spectrophotometric method considered below.

Several attempts to modify the Carr-Price method have been made and significant changes have included the use of catechol (*40*) and guaiacol (*41*). The test performed with guaiacol was claimed to provide greater specificity relative to carotene which might be present and to improve persistence of the color formed. The effect of 20 different modifications of the antimony trichloride reaction has recently been reported by Pacini and Taras (*38*).

Dann (1938) applied the Evelyn (*9*) photoelectric colorimeter to the antimony trichloride reaction. His contribution to the

technique, especially in the use of the photoelectric colorimeter, undoubtedly provides needed objectivity by eliminating errors due to personal judgment. While the dependability of such a method may be established for a certain type of material, the possibility of interfering substances in other products may still be significant. Also, speed and special precautions are still needed, though undoubtedly not so urgently as they were originally.

Ultraviolet Absorption

Essentially the only physical characteristic of vitamin A utilizable for its determination is its absorption in the ultraviolet at 328 mμ. It appears that the 328 mμ ultraviolet absorption characteristic was first discovered by Takahashi in 1925 in fish liver oil concentrates (*46*). Subsequent British investigators, starting with Morton and Heilbron in 1928, showed a correlation between absorption and biological activity (*7*, *12*, *32*, *35*).

The conclusion from early evidence largely accumulated by the British workers indicated the ultraviolet absorption to be a more reliable index of vitamin A content than antimony trichloride color reaction with its attendant complications. In 1934 the International unit of vitamin A was established by the Health Organization of the League of Nations as 0.6 microgram of beta-carotene. Since that time a large amount of effort both in England and in this country has been directed toward establishing a possible quantitative relationship between biological units and the unit of absorption at 328 mμ which has come to be known as the $E^{1\%}_{1cm.}$ value. This problem has been the subject of individual studies as well as collaborative effort both in this country and in England (*1*, *23*, *26*, *27*). It is now ordinarily assumed that the vitamin A ultraviolet absorption follows Beer's law in concentrations suitable for spectrophotometric examination. Experience in this laboratory, as well as reports and collaborative data from other laboratories, has indicated the validity of this assumption whenever certain precautions are taken, such as the maintenance of purity of the measured radiation. Generally, then, either the use of $E^{1\%}_{1cm.}$ value or a factor for converting $E^{1\%}_{1cm.}$ value into biological units is an accepted practice for comparing results. The factor may be established by direct comparison with biological assay or more generally by the use of a secondary standard whose biological value has been established. Such a secondary standard has been available in the U. S. P. reference cod liver oil biologically standardized against the International standard beta-carotene.

A curious lack of agreement between English workers and American workers as to what the conversion factor should be has persisted until recently. The British workers have found 1600 to be an acceptable factor, while in this country the value most generally used approximates 2000. Differences in biological procedures may possibly explain the discrepancies, although the question of a possible deterioration of improperly stored samples of the U. S. P. reference oil must be considered (*31*). The experienced British workers, Underhill and Coward (*8*), in 1939 deduced from biological studies involving crystalline vitamin A esters and the International standard beta-carotene that the conversion factor should probably be approximately 2000. The crystalline vitamin A prepared by Holmes and Corbet (*24*) was found by one of their collaborators to have a maximum $E^{1\%}_{1cm.}$ value of 2100. With a factor of 2000, this would mean that the pure crystalline vitamin A has a value approximating 4,000,000 units per gram. Biological evidence obtained with solutions of this pure vitamin A indicates that it does in fact have a potency of between 3,500,000 and 4,000,000 International units per gram.

The collaborative investigations have in general not yielded correlations as close as could be desired. The study completed in 1937 by the American Pharmaceutical Association yielded $E^{1\%}_{1\ cm.}$ values from 1.4 to 1.79 for the U. S. P. reference oil. The study completed last year by the American Drug Manufacturers Association yielded essentially the same values and the same amount of variation when both true spectrophotometers and modified ultraviolet absorption apparatus were jointly considered. However, when only the true spectrophotometers were used, the spread was reduced to ±8 per cent. At the present time another collaborative study by the U. S. Pharmacopoeia committee on the spectrophotometric vitamin A determination is nearly completed. This should afford a more acceptable conversion factor and some additional insight regarding previous difficulties, since this is the first study in which only true spectrophotometers standardized with potassium chromate have been exclusively used.

No doubt the greatest cause for discrepancies in either the ultraviolet absorption method or a colorimetric method lies in the labile nature of the molecule of vitamin A itself. Interrelationships with vitamin A_2 and other materials absorbing in the ultraviolet or interfering with a color test furnish additional complications.

Morton, Webster, and Heilbron treated a vitamin A concentrate in alcohol with hydrochloric acid and were able to obtain from the resulting solution the derived product which they identified as 1,6-dimethylnaphthalene. From this it was concluded that vitamin A had undergone cyclization (*13*, *15*, *22*). This change was associated with the development of numerous bands in the ultraviolet region. It appears that this degraded material may be formed in an oil under various normal conditions, especially if fatty acids are present, or it may occur naturally (*29*). The blue values for vitamin A are not changed by cyclization. The cyclized material has considerable ultraviolet absorption with the principal peak shifted to the region of 368 mμ. Some workers in this country have referred to this material as spurious vitamin A, since it has no biological activity.

A compound very similar to vitamin A_1 has recently been described and is known as vitamin A_2. Lederer and Rosanova (1937) found substances in certain Russian fresh-water fishes in which antimony trichloride bands at 690 and 645 mμ predominate (*29*). This finding was confirmed and elaborated in 1938 by Edisbury, Morton, Simpkins, and Lovern (*14*), Gillam, Heilbron, Jones, and Lederer (*19*), and Lederer and Rathman (*28*). Vitamin A or A_1 has a peak around 620 or 605 mμ in oils low in vitamin A and a direct absorption maximum at 328 mμ, while vitamin A_2 is responsible for a 690 to 697 mμ antimony trichloride band as well as one at 640 mμ. It was also found to possess direct absorption at the correspondingly longer ultraviolet wave length of 345 to 350 mμ together with another band in the region of 290 mμ. Gillam *et al.* judged that vitamin A_2 had the same formula as vitamin A_1 except that it had two or more carbon atoms or one more ethylene group. Contradictory evidence has been recently obtained (1939) by Gray to indicate that the differences may be explained by the presence of merely one additional double bond and that vitamins A_1 and A_2 therefore have the same number of carbon atoms (*20*).

Although vitamins A_1 and A_2 differ considerably in their ultraviolet absorptions and antimony trichloride values (*19*, *20*), the absorptions of the cyclized compounds have been found by independent investigators to be identical. However, it would appear that these cyclized compounds are not identical, since Embree and Shantz (*16*) have proposed a quantitative determination of each of the constituent vitamins when mixed, based on the differences of their respective adsorptions on alumina. Vitamin A_2 is more strongly held by adsorbing agents. Wald has proposed another method for the estimation of these two vitamins, using simultaneous equations based upon the differences found in the antimony trichloride absorption maxima (*47*).

Fortunately vitamin A_2 preponderates in fresh-water fish while vitamin A_1 preponderates in salt-water fish, although there appear to be some cases where both vitamins may be present in significant amounts. Thus a more satisfactory technique for the estimation of these two compounds in the presence of each other would be helpful.

Stability of Vitamin A

The stability of vitamin A is influenced principally by oxidation and by light. Vitamin A is destroyed rather easily by oxidation and certain substances, which appear to occur in

natural oils, can inhibit this oxidation. Also, the naturally occurring esters appear to be resistant to attack. It is common observation that an air space in the top of a bottle of fish liver oil can cause a very significant drop in strength in a few weeks, as evidenced by a decrease in the 328 mμ absorption and a corresponding decrease in biological potency even though the oil is stored in the dark in a refrigerator. The oxidative deterioration is distinguished by a decrease in absorption at 328 mμ without the development of new bands as in the case of cyclization.

With reference to the effects of light, some investigations (*6*, *10*, *11*) have shown that irradiation can partially destroy vitamin A, while others have shown that it can totally destroy it. Consequently, attempts have been made to determine vitamin A by measuring absorption before and after irradiation. The method seems to lack confirmation, although the possibilities in this direction do not appear to be exhausted.

Smith (*42*, *44*) and associates have reported some interesting studies concerning the stability of vitamin A in solution. They found that decreases in 328 mμ absorption caused by irradiation were partially reversible, indicating isomeric equilibrium affected by light. They also reported similar reversible changes by specific solvent action. For example, in one experiment a vitamin A–containing material was first made to volume in chloroform, the absorption was measured, the chloroform was removed with a vacuum, and the material was again made to volume with diethyl ether. In this experiment the latter ether solution was found to have an appreciably higher absorption value than the original chloroform solution. Such results have been attributed to isomerism. It would appear expedient to have in mind the possibility of such phenomena occurring inadvertently before or during a spectroscopic examination.

In the preparation of vitamin A solutions sufficiently pure for light-absorption measurements, it is ordinarily necessary to use extraction and saponification methods (*43*, *48*). (A fish liver oil, for example, might be saponified for 20 minutes with alcoholic potash, extracted four times with diethyl ether, then washed to remove soaps. The ether would then be evaporated almost to dryness but never to dryness, and made to volume with a solvent more suitable for spectrophotometric use.)

These methods have received considerable study and although not completely satisfactory at the present time, they are usually dependable for most materials in the hands of experienced workers. The fact that maximum correlation in collaborative work has not been attained with the nonsaponiable and extracted portions of oils suggested that further improvement in these methods is desirable. In the separation of carotene from vitamin A in solutions to be used for light-absorption measurements, both the selective action of solvents and the adsorption of carotene on charcoal have been reported to be satisfactory. Attempts have also been made to separate vitamin A by means of selective adsorption. In general, however, this may be a dubious practice since there is some evidence that chromotographic separation results in uncertain molecular changes in the vitamin component (*5*, *5*).

The evidence thus far accumulated indicates that quantitative measurements of absorption at specific wave lengths either before or after chemical reaction can be fairly satisfactory for a given product such as cod liver oil. On the other hand, it is just as evident that more complicated procedures are both possible and necessary for more accurate analytical work with miscellaneous vitamin A–containing materials. For such a program a more rapid and accurate definition of entire spectral curves throughout the ultraviolet and visible regions is regarded as fundamental. The author is therefore at present actively concerned with apparatus, and while his work in this direction is probably far from complete, he feels that progress is being made along this line. It is expected that such apparatus will be the subject of subsequent publication.

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Recent Developments in Methods for Determining Carotene

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MOST procedures that have been devised for the determination of carotene and other carotenoid pigments have been based upon the discovery of Borodin (*3*) in 1883 that the carotenoid pigments could be separated into alcohol-soluble and petroleum ether–soluble fractions. Early methods for the quantitative determination were also reported by Arnaud (*1*) and by Monteverde and Lumbimenko (*20*), but the method which has been used most widely as a starting point in the development of new techniques is that of Willstätter and Stoll (*36*).

Lest it be assumed that the latter method, which was published in 1913, is only of historic significance scientifically it should be pointed out that in most essentials later extraction methods have offered no strikingly new contributions. The extent to which this is true is shown by the following brief summary of the Willstätter and Stoll method.

The fresh plant material is ground finely in a mortar with quartz sand and 40 per cent acetone. The ground material is then filtered and washed with 30 per cent acetone until the filtrate comes through clear. The extracted material is finally washed with pure acetone, removed from the filter, macerated once again under pure acetone, and filtered a second time. The combined acetone extract is then treated with ether and the acetone saponified with methyl alcoholic potash, which is removed from the ether solution by washing with water. The ether extract is evaporated to dryness with vacuum, the residue taken up in petroleum ether, and the extract poured into a separatory funnel. Xanthophyll is then removed from the carotene by washing first with 85 per cent methanol, then 90 per cent, and finally 92 per cent methanol until the washings are colorless. The xanthophyll which is present in the alcohol phase is then brought into ether solution. Both the carotene and xanthophyll solutions are washed free of methanol with water, dried and brought to volume and the concentrations are determined colorimetrically, using a 0.2 per cent solution of potassium dichromate as the colorimetric standard.

Numerous modifications of the Willstätter and Stoll method have appeared (*5*, *11*, *15*, *28*, *29*, *30*, *32*). Of these, the Guilbert method (*11*), which was first presented in 1934, has been most widely used as a starting point for recent modifications. In this method, ethyl ether is used as an extraction solvent from which the pigments are transferred to petroleum ether. Rapid and effective saponification and extraction are accomplished simultaneously by boiling the sample for 0.5 hour with saturated alcoholic potash.

More recently Peterson, Hughes, and Freeman (*26*) introduced a modification of the Guilbert method which is considerably shorter, eliminates several possibilities of carotene loss in manipulation, and gives results which are readily reproducible. The original ether extraction of the Guilbert method has been eliminated entirely in their modification. Instead, petroleum ether (boiling point 40° to 60°) is used. This obviates the necessity of carrying on a single solvent evaporation during the course of the determination, and excludes the possibility of carotene decomposition which might occur during the ether evaporation required in the original method. The method is considerably shortened, inasmuch as the chlorophyllins, flavones, alkali, and xanthophyll can be removed directly from the petroleum ether exactly as Guilbert describes their removal from ether and petroleum ether, respectively.

Since the latter modification was published a number of changes have been made in the method. Some of these changes were a development resulting from experience gained in the carotene analyses of several hundred samples yearly. Other changes were suggested by published modifications.

Early in the author's investigations it was found, for example, that the original method, which was highly effective in the extraction of carotene in dry feeds which had been run through the Wiley mill, extracted only 50 to 80 per cent of the total carotene from fresh tissues such as pasture plants, grasses, silages, etc. With these materials it was found advisable to grind the residue from the original digest under alcoholic potash with sea sand in a mortar and to reflux again for an additional 15 minutes to remove all the carotene. If the residue is only ground under alcohol but not redigested, 95 per cent of the carotene is removed. If, instead of grinding after the original digestion, the sample is ground before digestion the extraction is not so complete, usually 90 to 95 per cent of the carotene being extracted. It is always a safe practice in the carotene analysis of strange materials to repeat the digestion of the thoroughly macerated residue to determine whether all the carotene has been removed by one extraction. This technique has also proved valuable in testing the efficacy of other extraction techniques which do not involve the use of alcoholic potash as an extraction and saponification medium.

In the original method of Peterson, Hughes, and Freeman, it was customary, after digestion of the sample was complete, to decant the liquid extract from the sample residue into a separatory funnel. The residue was re-extracted several times with petroleum ether and the solvent similarly removed. This operation, from a routine standpoint, was slow, and with finely ground meals stopcocks of the separatory funnels were frequently clogged. Sintered-glass filter funnels were found to be excellently suited to this step in the procedure, since the residue can be repeatedly and conveniently washed and stirred on the funnel plate until the solvent comes through clear. Alcohol and petroleum ether, used alternately in small portions, are particularly effective. The use of Büchner funnels fitted with the appropriate paper is also an improvement over the older decantation technique.

At the suggestion of Buxton and Dombrow (*4*) it was found that chlorophyllins, flavones, alkali, and xanthophylls could be removed from the original petroleum extract effectively by direct extraction with 90 per cent methanol. It is not necessary to remove first the water-soluble constituents by preliminary washings with water. This also reduced appreciably the number of washings with 90 per cent methanol, since it appears that xanthophylls are more soluble in the presence of small amounts of alkali. In the extraction of xanthophylls, no advantage can be found in extracting the petroleum layer with 85 per cent methanol preliminary to washing with 90 per cent methanol.

Since most petroleum ethers ordinarily used in the extraction of carotene are partially soluble in 90 per cent methanol, considerable difficulty is frequently encountered in the serious reduction of volume in the petroleum phase during the removal of the xanthophylls. Skellysolve B, a widely used commercial grade of petroleum ether, has a solubility of 12 cc. per 100 cc. of 90 per cent methanol. Where this mutual solubility of the two solvents is troublesome it is advisable to saturate the methanol with petroleum before use.

Revised procedures may now be presented for the extraction of carotene from dry and fresh plant tissues.

Extraction of Carotene from Dry Plant Tissue

Weigh out the samples (1 to 5 grams, more or less, depending on the relative potency), transfer to a 250-cc. Erlenmeyer flask, and add 100 cc. of freshly prepared, 10 per cent ethanolic potassium hydroxide. Fit the flasks with reflux condensers, and boil the contents on a steam bath or hot plate for 30 minutes. If portions of the sample collect on the sides of the flask, wash down

with alcohol from a wash bottle. Cool the contents of the flask, then pour them into a sintered-glass filter funnel, applying a vacuum only until most of the solvent has come through. Then wash the residue alternately with 25-cc. portions of Skellysolve B and absolute alcohol until the filtrate comes through clear. The suction should at no time be applied unless the sediment is partially covered by solvent. After the addition of each wash portion of solvent, more complete extraction may sometimes be obtained by stirring the sediment on the funnel plate with a stirring rod before applying suction. Transfer the filtrate to a 500-cc. separatory funnel.

Pour gently about 100 cc. of distilled water through the alcohol-Skellysolve solution in the separatory funnel. Draw off the alkaline alcohol-water solution from the bottom of the funnel, and re-extract three times by shaking gently with 30-cc. portions of Skellysolve B, using two other separatory funnels. Combine the Skellysolve extracts and wash free from chlorophyllins, flavones, alkali, and xanthophylls by shaking thoroughly with 30-cc. portions of 90 per cent methanol (five washes are generally sufficient) and re-extract the first methanol portion with 50 cc. of Skellysolve B. Wash the Skellysolve B solution once with 50 cc. of distilled water to remove the alcohol and filter into a volumetric flask through filter paper upon which is placed a small amount of anhydrous sodium sulfate. After making the carotene solution up to a definite volume, determine the concentration by the spectrophotometer, photoelectric colorimeter, or colorimeter by comparison with 0.1 per cent or 0.036 per cent potassium dichromate.

Extraction of Carotene from Fresh Plant Tissue

Cut fresh plant tissue finely with shears and mix as thoroughly as possible. Weigh samples of 4 to 10 grams into a 250-cc. Erlenmeyer flask and proceed with digestion and washing as for dry materials. When the residue on the filter plate has been thoroughly washed, transfer it into a deep mortar with a well-defined lip, add 10 cc. of a saturated solution of potassium hydroxide in ethanol, and macerate with the pestle. Add quartz sand and grind. From time to time wash down the sides of the mortar with a stream of alcohol from a wash bottle and grind until the tissue is fine. Transfer the contents of the mortar to the Erlenmeyer flask used in the original digestion, using more alcoholic potassium hydroxide to effect the transfer. Add alcoholic potassium hydroxide until the total volume is 100 cc., digest for 15 minutes, and proceed as indicated for dry tissue. Combine filtrates from both digestions and washings before extraction of xanthophylls.

Determination of Carotene Concentration

Of the three methods available for the determination of carotene concentration, colorimetric, spectrophotometric, and photoelectric photometric, the colorimetric method has probably been used most extensively (*1*, *11*, *21*, *26*, *27*, *28*). Accurate results have been obtained by this method. The spectrophotometer, however, has an additional advantage in that no standard solution is needed for comparison. One needs only determine the absorption coefficient for pure carotene in the solvent to be used at some convenient wave length, usually in the region of one or both of the absorption maxima.

In laboratories of this station, as in many others, the photoelectric colorimeter (*31*) has almost completely replaced the colorimeter and spectrophotometer in the determination of pigment concentration.

The spectrophotometer, however, has not lost its value in the study of many aspects of the carotene problem. It is useful in the determination of related chromogens which frequently are found in carotene extracts, and which may or may not possess vitamin A potency, and may also be used to advantage in the calibration of the photoelectric photometer. It is in the latter use of the instrument that some significant observations regarding carotene solutions have been made.

The photoelectric photometer is usually calibrated for the determination of carotene by the preparation of a curve of transmission *vs.* carotene concentration in which the carotene concentration of a number of solutions has been determined or checked by means of the spectrophotometer. Crystalline β-carotene, which gives a normal absorption spectrum, is usually the standard. Rarely, however, does one find in practice a carotene extract which has absorption maxima and a minimum identical with that of true β-carotene, nor are the ratios of the optical densities at various wave lengths correct. The value of $\log \frac{I_0}{I}$ at 4500 Å. is frequently too large with respect to that at 4700 Å. (*27*) while $\log \frac{I_0}{I}$ at 4800 Å. is usually less than that at 4700 Å. In Table I are shown the relative spectral absorptions of a number of carotene extracts from different sources expressed in percentages of absorption at wave length 4700 Å.

Table I. Relative Spectral Absorption of Carotene Extracts

(Expressed in percentages of absorption at wave length 4700 Å.)

No. of Samples	Sample	4500 Å.	4700 Å.	4800 Å.
5	β-Carotene	118	100	105
20	Spring grasses	117	100	103
21	Summer grasses	119	100	103
11	Fall grasses	129	100	106
6	Butter	114	100	104
42	Egg yolk	117	100	92
46	Dehydrated alfalfa	119	100	105
16	Prairie hay	138	100	103
3	Sorghum silage	123	100	100
6	Yellow corn	116	100	87
27	Miscellaneous commercial feeds	120	100	96

It is apparent from an inspection of Table I that a carotene concentration obtained by means of a photoelectric photometer, calibrated with true β-carotene, may agree with that obtained by the spectrophotometer at one wave length, and not at all at some other wave length.

Reasons for these peculiar changes in the absorption spectrum of β-carotene are not readily explained. On all adsorbents the main pigment fraction adsorbs in the same position as β-carotene and cannot be distinguished from it when the two are adsorbed simultaneously. Within the usual limits of experimental error in the rat assay for vitamin A potency it appears that the main pigment fraction has a vitamin A potency equivalent to that of true β-carotene. The main pigment fraction on removal from an adsorption column possesses an abnormal absorption spectrum approaching more nearly that of α-carotene rather than β-carotene. Zechmeister and Tuzson (*38*) have made the observation that carotenoids readily undergo certain isomerization processes in certain solvents, particularly when heated. These changes are said to be accompanied by a movement of the extinction maxima toward the shorter wave lengths. It is claimed that this phenomenon is not induced by the adsorption process as described by Gillam and co-workers (*10*). All attempts to separate petroleum fractions from a number of the materials listed in Table I under conditions known to prevent the solvent, heat, and adsorption effects described resulted in no improvement in the absorption spectra. It seems clear that the abnormal absorption spectra are an intrinsic property of the pigments as found in the original material previous to extraction.

No discussion of methods for the determination of carotene would be complete without some consideration of techniques suggested for the separation of "true" β-carotene from the noncarotene chromogens found in most petroleum extracts of feeds and silages.

Hegsted, Porter, and Peterson (*12*) have described a method involving the extraction of the petroleum extract with aqueous diacetone.

This technique, which can be applied successfully in the separation of acid-modified carotenoids present in silages, has not proved generally applicable with many other forages. Attempts to use this solvent in the extraction of cryptoxanthin from petroleum extracts of yellow corn have also proved unsuccessful. It is unfortunate that selective solvents have not been more successful in the separation of these impurities from petroleum ether solutions, since this would offer a convenient and rapid method of solving this troublesome problem. In view of the similar solubilities it seems very unlikely that absolute separation of such closely related pigments will ever be accomplished by phase separations.

FIGURE 1. CHROMATOGRAPH OF PETROLEUM-PHASIC FRACTION OF CANADIAN YELLOW CORN ON A MAGNESIA COLUMN
Left. Improperly washed tube
Right. Properly washed tube

Adsorption methods have proved by far the most successful in the purification of carotene solutions. Fraps and associates (*6–9*) have recently studied extensively methods of selective adsorption.

Their technique involves shaking the impure carotene solution with a given amount of specially activated adsorbent (magnesium hydroxide or carbonate). These authors have also described an "adsorbent X" which adsorbs xanthophyll but does not absorb carotene, and an "adsorbent L" which removes large quantities of impurities in such foods as watermelon, ripe tomatoes, and apricots. The latter impurities are not removed by "adsorbent X". In a recent contribution, Fraps, Kemmerer, and Greenberg (*9*) have presented a new modification of the modified A. O. A. C. method (*21*) in which xanthophylls and impurities are adsorbed directly instead of first washing with methyl alcohol and then using the adsorbent.

With the idea of providing suggestions for further improvements in this direction, the following points may be presented in criticism of the present methods of Fraps *et al.*: (1) special techniques are required in the preparation of the adsorbents; (2) proper activation of the adsorbent must be frequently checked to determine whether or not it will adsorb the desired impurities and not β-carotene; (3) completeness of adsorption must be checked photocolorimetrically from time to time throughout the determination until no more color is removed; (4) no provision is made for determining the quantity or number of pigments adsorbed or for studying their absorption spectra and other properties; (5) the colored impurities associated with carotene may not be identical in all feeds or in different samples of the same feed, thus involving the preparation of an adsorbent of a different degree of activation; and (6) the vitamin A potency of these impurities has not been extensively investigated.

Chromatographic Adsorption

There probably is no development which has been so helpful in the separation and identification of the closely related carotenoids as has chromatographic adsorption, or the separation of pigments by their adsorption on a column of adsorbent suited to the purpose.

Since the early work of Tswett (*34*) adsorption methods have been used to advantage and improved techniques have been developed by a great many workers in the field, notably Palmer (*22, 23*), Palmer and Eckles (*24*), Vegezzi (*35*), Lipmaa (*18*), Kuhn and Lederer (*16*), Karrer and Walker (*13*), Kuhn, Winterstein, and Lederer (*17*), and Kuhn and Brockmann (*14, 15*).

Especially noteworthy is the work of Strain (*33*) who, in the study of numerous adsorbents for specific purposes, has found that a special brand of magnesium oxide possesses the most desirable qualities for the resolution of different carotenes.

Precautions and conditions which must be observed in the preparation and effective use of adsorption columns have been described by Miller (*19*) and by Zechmeister (*37*).

Though chromatographic adsorption techniques have been very useful in the qualitative separation of the fractions present in a pigment mixture and have permitted their identification, attempts to apply adsorption methods on a strictly quantitative basis have been only partially successful. An adsorbent which is sufficiently activated to accomplish a sharp and clear-cut separation of the component fractions of a pigment mixture may hold the pigments so tenaciously as to make their removal from the adsorbent difficult. Oxidative effects are also likely to occur. On the other hand, an adsorbent which is less activated, and permits a more nearly quantitative removal of adsorbed pigments, is apt to have lost much of the power of selective adsorption frequently necessary in the separation of very closely related carotenes.

Chromatographic adsorption studies covering a period of three years on the petroleum-phasic fractions of a large variety and number of feeds and biological materials using the Strain technique have given an average recovery of only 84 per cent.

COLUMN PREPARATION. Further refinements in the technique of column preparation have been developed.

Unless more than the usual care was taken in assuring the cleanliness of the tube before introducing the adsorbent, a washing or seepage of pigment away from the main pigment zones along the inner glass surface occurred. This could be prevented by preliminary treatment of the tube with dichromate cleaning solution. In Figure 1 is shown a chromatographic separation of β-carotene and cryptoxanthin in Canadian yellow corn, on a column in which the tube had not been treated with cleaning solution before packing, and the same separation in a cleaned tube.

A new technique was developed in the preparation of adsorption columns as a result of attempts to obtain acceptable photographs. In all earlier attempts the chromatographs showed marked "packing lines" where separate portions of added adsorbent had come together in the packing of the column. Apparently the lower part of each disk of adsorbent was less densely packed than that part which had come in direct contact with the packing tamper. Thus, in the development of a chromatograph that region immediately above a packing line adsorbed considerably less pigment than that region immediately below, making identification of the true pigment zones difficult. In Figure 2 is shown a chromatograph of the xanthophylls of a molasses–oat grass silage, illustrating to advantage the confusing effects of packing lines.

FIGURE 2 (*Left.*) CHROMATOGRAPH OF XANTHOPHYLLS OF MOLASSES–OAT GRASS SILAGE

From dichloroethane solution of pigments on magnesia columns, showing packing lines due to improper preparation of column

FIGURE 3 (*Right*). CHROMATOGRAPH OF PETROLEUM-PHASIC FRACTION OF ARGENTINE YELLOW CORN ON MAGNESIA COLUMN PROPERLY PREPARED

Though it proved difficult to prepare an adsorption column which did not show slight evidences of packing lines it was possible greatly to minimize the effects of these lines on the proper development of a chromatograph. This was accomplished by packing only small portions (0.63 to 1.25 cm., 0.25 to 0.5 inch) of adsorbent at a time in building up the column, and, after the uniform packing of each disk, loosening the upper, tightly packed portion (0.156 to 0.31 cm., 0.063 to 0.125 inch) by means of a square-ended spatula before packing the next portion. A column, so prepared, is shown in Figure 3.

Petering-Wolman-Hibbard Procedure for Determining Carotene

Of recent methods for the determination of carotene, one of the most interesting and promising is the novel method of Petering, Wolman, and Hibbard (*25*), designed primarily for fresh tissue.

The finely cut tissue is ground with quartz sand and acetone repeatedly with mortar and pestle until the solvent comes off clear. The solvent is brought to volume and an aliquot is boiled with a small amount of activated barium hydroxide or barium hydroxide octahydrate. The solution is filtered by suction, the barium hydroxide retaining the chlorophyll by adsorption. The xanthophylls and flavones are separated from carotene by the usual methods.

If the proper precautions are taken, excellent results may be obtained by this method. The barium hydroxide must be free of carbonate and must be finely dispersed. A highly active preparation may be obtained by filtering 15 cc. of a hot saturated aqueous solution of barium hydroxide directly into the acetone extract immediately before digestion. This obviates the necessity for keeping on hand a barium hydroxide strictly free of carbonate. The aliquot must also be sufficiently small that the chlorophyll it contains can be completely adsorbed by the amount of barium hydroxide recommended.

Benne, Wolman, Hibbard, and Miller (*2*) have recently published the results of a comparison of the above method and two modifications thereof with the technique of Peterson, Hughes, and Freeman (*26*). A large variety of plant tissues were analyzed, and the results were comparable throughout, except with certain samples of bluegrass taken late in the season. Similar comparisons in this laboratory have shown that the greatest discrepancy lies in the inefficacy of the grinding method for removing all the carotene from fresh tissue. Excellent checks were always obtained by the two methods when aliquots of the filtrate were used as the starting point. There is evidence that grinding until the solvent appears clear does not always ensure complete extraction. It is the opinion of the author that the alcoholic potash digestion method can effectively replace much of the manual labor of grinding in the disruption of tissue.

Though it is not believed that the barium hydroxide technique offers many advantages with regard to the time required in effecting a complete analysis, it does have several valuable features. For example, when chlorophyll is once removed, all the remaining carotenoids can be transferred quickly and completely to the petroleum phase. This is not the case in the older method, since xanthophylls are but slowly removed from alcoholic potash, and, to the uninitiated, it is difficult to determine when one is removing carotene and xanthophyll, or only xanthophyll from the alcoholic potash mixture. This method should have advantages in the determination of cryptoxanthin and many other carotenoids which are less readily removed from alcoholic potash solutions by means of petroleum ether.

Summary

Improvements in methods for the extraction and quantitative determination of β-carotene in dry and fresh plant tissue are described.

Solvent and adsorption methods for the separation of β-carotene from accompanying petroleum-soluble carotenoids are discussed in the light of new developments.

The Petering-Wolman-Hibbard method has excellent potentialities in the development of methods for the quantitative determination of cryptoxanthin and other carotenoid pigments, as well as carotene.

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Chemical Methods for the Determination of Vitamin B_1

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THE importance of rapid and accurate methods of analysis for vitamin B_1 needs no emphasizing. The biological methods have unavoidable limitations to which the chemical methods are not subject. The nonbiological methods for the determination of vitamin B_1 are based on the measurement of the concentration of substances produced from the vitamin in standardized procedures. Although the percentage conversion of thiamin into the measured product is fairly constant in the concentration range used in these methods, in no case is the vitamin known to be so converted either completely or exclusively.

The methods of Kinnersley and Peters (*15*, *16*), of Willstaedt and Barany (*40*), and of Melnick and Field (*20–24*, *26*), the last-named using a reagent proposed by Prebluda and McCollum (*28*, *29*), all depend on the formation of a pigment, presumably formed by the coupling of the vitamin with a diazotized aromatic amine. The measurement, by colorimetric or photometric means, of the amount of pigment produced becomes a measure of the amount of vitamin B_1. The aromatic amines employed in these methods by these investigators are sulfanilic acid, 2,4-dichloroaniline, and *p*-aminoacetophenone, respectively. Recently, Emmett, Peacock, and Brown (*4*) have simplified the Melnick and Field procedure in its application to certain materials having a fairly high potency.

A method of vitamin B_1 assay based on a type of reaction and measurement different from those described above is proposed by Jansen (*14*). Thiamin is oxidized in alkaline medium by ferricyanide and thus converted into thiochrome. When irradiated by light in the near ultraviolet region of the spectrum, this substance shows a strong blue-violet fluorescence, the intensity of which is proportional to the concentration of the thiochrome. This, in turn, is proportional to the amount of thiamin from which it was formed by oxidation. Extraction of the thiochrome from the oxidation medium by isobutanol increases the intensity of the thiochrome fluorescence, while at the same time the aqueous layer retains most of the interfering materials. A blank determination for the measurement of preformed nonspecific fluorescence is conducted with the omission of the ferricyanide. Adsorption on Franconite with subsequent elution and oxidation is suggested for urine.

This general procedure has been used with or without modification by many investigators. The more important earlier references are given by Hennessy and Cerecedo (*10*). Many later publications have appeared, some describing modification of the method in its details or application to different materials. The method has been applied to the analysis of blood by Jansen (*13*) Widenbauer (*36*), Widenbauer *et al.* (*37*), and Ritsert (*31*, *32*) Thiamin has been determined in urine by several investigator (*3*, *6*, *11*, *18*, *26*, *30*, *33*, *35*, *38*). More general application of th method is described by Hennessy and Cerecedo (*10*), Wiegan (*39*), Bertagni (*2*), and Flavier and Genevois (*7*). Some investi gators are critical of the procedure as employed with variou modifications by others. In particular, Wang and Harris (*35*) Hills (*11*), Mallinckrodt-Haupt (*19*), Otto and Rühmekorb (*27*) Huhn (*12*), Baucke (*1*), and Gahlen (*8*) have suggested deficien cies, usually as a prelude to offering some alternative.

The first problem in any of the chemical methods is to ob tain the vitamin in solution, and this in many cases require an extraction procedure. Whether or not complete extrac tion may be expected with all types of materials is not defi nitely known. Refluxing and heating in a boiling water bat with 0.1 *N* sulfuric acid have been practical means of extrac tion. A single extraction suffices, especially if the ratio o the volume of extracting liquid to the weight of sample i maintained as high as is feasible in the light of the minimun concentration of thiamin required in the aliquot of extract t be used in the subsequent step of the assay procedure. A ratio greater than 50 cc. of extracting liquid per gram o sample is generally not necessary, while if this ratio be les than 15 to 1, a lowered extraction efficiency may result. In cubation of the residues of a properly conducted extractio with various digestive enzymes has not shown any significan quantities of vitamin B_1 remaining therein. General agree ment of the results of chemical assays of extracts with the re sults of biological assays of the corresponding samples woul indicate that the extraction is as efficient as the process takin place in the digestive tract of the animal. In the literatur (*4*, *10*) this is shown to be the case with many materials When a chemical and biological assay of a sample of som material agree with each other, this agreement may be ex pected to persist for other samples of the same material.

The occurrence of thiamin, in some sources, in the form o its phosphoric esters necessitates an enzymatic hydrolysis which usually follows immediately after the extraction Takadiastase, mylase, clarase, diastase-Merck, kidney phos

phatase, and yeast phosphatase have been used to bring about this hydrolysis. The first four of these, being diastatic enzymes, are particularly suitable for use in the preparation of extracts of starchy materials. Takadiastase has been most widely employed. Maintenance by a buffer of the pH at the optimum for the phosphatase activity is essential during the entire incubation period if the hydrolysis is to be completed within the allotted time. The need for the conversion of the thiamin esters into thiamin may be found in the consideration of their different behavior in the purification step, or in the solvent extraction step, both of which are used in the two types of chemical methods. In fact, thiamin in the form of its phosphoric esters may be completely lost in these steps.

Any chemical method of analysis of a substance occurring in its source in 1 to 2000 parts per 10,000,000, unless it be highly specific, must allow considerable purification without losses before the actual colorimetric or fluorometric procedure is begun.

The most successful single step which can bring about the desired purification is that proposed by Hennessy and Cerecedo (*9*) for use with the thiochrome method. Melnick and Field (*24*) used a similar step in their colorimetric method. It consists of passing the impure extract which contains the vitamin through a column of the synthetic zeolite, Decalso. The vitamin is retained on the zeolite, from which it can later be removed by treatment of the zeolite column with potassium chloride solution. This process is not essential for the thiochrome procedure when materials of fairly high potency are being assayed. The variable and incomplete recoveries, the high blanks, and the need for varying the ferricyanide concentration in the thiochrome method, described by the European investigators who used Franconite or similar clays for purification, are not experienced when the base-exchanging zeolite is employed. Melnick and Field report that the use of clay is not satisfactory as a method of purification in their procedure. Ritsert and Wang and Harris have omitted the use of Franconite in determining vitamin B_1 in urine by the thiochrome method, as they believe that the benefits accruing from the use of the adsorption step are outweighed by the disadvantages.

An analysis of the sensitivity of the colorimetric and the fluorometric methods shows that the advantage lies with the latter, particularly in the assay of low-potency materials.

The amount of thiamin in the aliquot, which is used to produce the pigment in the colorimetric methods, should be more than 5 micrograms, the preferable range being 20 to 100 micrograms. For the fluorometric thiochrome procedure, the aliquot need contain only 0.1 microgram with the preferred range between 0.5 and 2.5 micrograms of thiamin. For general practicability, it is essential that a fluorometer, if used, be of sufficient sensitivity to provide a galvanometer deflection of at least five scale divisions for 0.1 microgram of thiamin chloride. The Pfaltz & Bauer fluorophotometer (*25*) meets this requirement. The usual type of colorimetric or photometric instruments may be used as the means of measurement of the pigment produced in the Melnick and Field procedure. It is only necessary that they be adaptable to a 2-cc. volume.

The reproducibility of the methods is of the same order. In a series of determinations, one may expect an average deviation of less than 5 per cent.

Of the colorimetric procedures, that of Melnick and Field has had its specificity best established, although the exact chemical basis of this specificity is not agreed upon by Prebluda and McCollum (*29*) and Melnick and Field (*20*). The chemical composition and structure of the pigment are not known. A well-established fact, which as yet has no explanation, is the effect of phenol and alcohol on the reaction between thiamin and the Prebluda-McCollum reagent. Melnick and Field add these materials to the reaction mixture in order to increase sensitivity, stabilize the vitamin, eliminate variation in apparent recovery, eliminate the effect of trace sensitizers, and permit the use of a single standard. The reaction is allowed to proceed overnight to give maximum color production. There is a possibility of a shorter reaction time, since a high percentage of the final color intensity is produced in less than 0.5 hour.

The results obtained by Melnick and Field and by Emmett and co-workers on materials of relatively high potency have shown good agreement with the results of biological assay. The Melnick and Field procedure for the assay of urine (*23*) has given results in substantial agreement with the results of the thiochrome assay on the same samples. The results of the thiochrome method were on the average 15 per cent higher than those of the Melnick and Field procedure on six samples of urine ranging in potency from 50 to 1300 micrograms per 24-hour sample (*5*). This is reassuring, since the two procedures are based on entirely different reactions. The less sensitive colorimetric procedure requires a 4- to 8-hour sample (75 to 300 cc.), while the fluorometric method needs only 10 to 20 cc. of urine. With the larger quantity of urine used in the colorimetric procedure, removal of salts by a benzyl alcohol treatment is needed to ensure nearly complete recovery of thiamin at the zeolite purification stage (*23*).

Thiochrome Method

The specificity of the thiochrome method is based on extensive comparison with the results of bioassay on a variety of materials of the entire range of potency (*10*). The chemical basis is well known, since the chemical structure of thiochrome has been established (*17, 34*). The oxidation of thiamin in alkaline ferricyanide solution does not seem to result in the production of an equivalent amount of thiochrome, but whether this is due to the establishment of an equilibrium in the oxidation process or to side reactions is not definitely known. Nevertheless, under the conditions of the method, when interfering materials are efficiently removed, the production of thiochrome proceeds uniformly. The phosphoric esters of thiamin, when oxidized, form what are probably the phosphoric esters of thiochrome. Measured in the aqueous alkaline oxidation medium, the fluorescent intensities of the three thiochromes are in the ratio 12 to 15 to 19 on an equivalent basis in the order of increasing phosphoric acid content. This precludes any direct measurement of mixtures of the esters by fluorometry on the aqueous phase following isobutanol extraction of thiochrome.

In the thiochrome method, a blank determination is made for the measurement of preformed, nonspecific fluorescence by omitting the potassium ferricyanide. In most cases, the zeolite purification eliminates or reduces to insignificance the materials responsible for this fluorescence. There is the possibility that these materials, if present, may be partly destroyed when the potassium ferricyanide is used for the oxidation of thiamin to thiochrome. The difference between the readings obtained with and without oxidation would be erroneously low in this case. This consideration is of importance only if the nonoxidized reading be larger for the sample than for the standard thiamin chloride by an amount of 10 per cent or more of the difference between the oxidized and nonoxidized readings for the sample. In such cases the same nonspecific fluorescence which is destroyed by the ferricyanide also largely disappears merely on standing for 5 minutes with alkali and isobutanol (*5*).

The thiochrome method is being used routinely in this country with considerable success and satisfaction in many industrial, clinical, and university laboratories. The procedure now in general use is essentially that of Hennessy and Cerecedo (*10*) with subsequent changes reported by Hennessy at the Baltimore, Boston, and Detroit meetings of the

American Chemical Society. The main purpose of these changes has been to develop a more suitable routine procedure.

Preparation of Extracts. The extraction is now accomplished in a single treatment of the sample with 0.1 *N* sulfuric acid. The pH of 4.0 to 4.5 required for the enzymatic hydrolysis and for the base exchange is attained by the addition of 0.1 volume of 1.2 *M* sodium acetate solution to the extraction mixture.

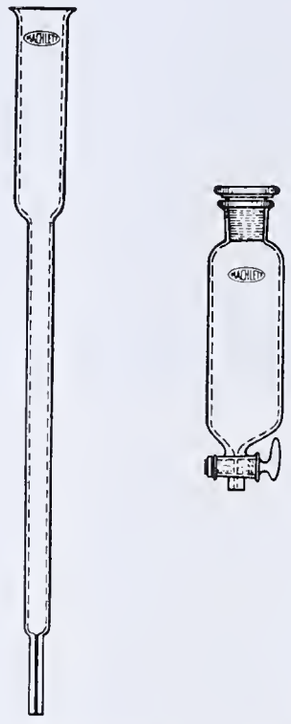

Figure 1. Separatory-Centrifuge and Exchange Tubes

Method I. All preparations, including the standard thiamin chloride, should be in 20 to 25 per cent potassium chloride solution at the oxidation step, to keep the volume of isobutanol constant in the extraction. It has been found advantageous to mix the ferricyanide and alkali before adding them to the sample. A separatory-centrifuge tube (obtainable from E. Machlett & Son, New York, N. Y.) has been devised (Figure 1, right), so that oxidation, extraction, separation, and clarification may be carried out in this single vessel with uniform timing throughout. The shaking time for the isobutanol extraction seems best at 1.5 minutes, followed by centrifuging at 500 r. p. m. for 0.75 minute.

Method II. *Exchange Tube.* The tubes for the exchange (Figure 1, left) are 15 × 0.7 cm. with a 25-cc. reservoir at the upper and a 3.0 × 0.03 cm. capillary at the lower end. A 6- to 7-cm. column of Decalso is held in the tube by a small plug of glass wool at the lower end.

The Exchange. The base exchange is conducted at room temperature, using 60- to 80-mesh Decalso prepared in bulk by stirring or shaking with four 10-volume portions of 3 per cent acetic acid for 10 minutes each. Between the second and third acid washing a 15-minute treatment with 5 volumes of 25 per cent potassium chloride solution is introduced. Thorough washing with water finishes the preparation of the Decalso. The eluting mixture is 25 per cent potassium chloride in 0.1 *N* hydrochloric acid. Complete removal of thiamin from the zeolite is accomplished by passing 21 cc. of this mixture at room temperature at a rate not exceeding 0.9 cc. per minute. This allows for four 5-cc. aliquots. As many as sixteen of these tubes may be run in parallel and eight samples carried through this step in duplicate in 70 minutes. Although the Decalso may be used repeatedly, it is safer practice to use a fresh charge for each sample.

Method III. *Enzymatic Hydrolysis.* The enzymatic hydrolysis is brought about in 2 hours at 50° C. using 0.5 per cent takadiastase at pH 4.0 to 4.5. This hydrolysis, if carried out on extraction mixtures, is done before any separation of the still insoluble portions is attempted.

Fluorometry. The use of quinine sulfate is to be preferred to the use of the rear photocell of the fluorophotometer for setting the galvanometer deflection per microgram of thiamin chloride. The quinine sulfate fluorescence should be determined immediately before that of the unknown.

The thiochrome method with the modifications described above has been applied to almost all the samples whose assay was reported by Hennessy and Cerecedo (*10*). The results are the same, but are obtained with less effort and in a shorter time. Other materials of all types have been assayed, the results being, with few exceptions, in good agreement with the results of biological assay.

Between eight and twenty samples may be assayed in duplicate in a single day by the thiochrome method.

Summary

Accurate and rapid chemical methods are available for the determination of vitamin B_1.

The Prebluda-McCollum reagent used in the manner prescribed by Melnick and Field is practical for such determinations on materials of moderate and high potency. The thiochrome method, having considerably greater sensitivity than the colorimetric methods, may also be used for the assay of materials of low potency. The use of the synthetic zeolite, Decalso, as a preliminary step in both procedures effectively eliminates interfering materials. Recent modifications of the thiochrome method have made it suitable for routine analysis.

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Determination of Vitamin B_2 (Riboflavin)

Comparison of Bioassay, Microbiological, and Fluorometric Methods

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THE increased interest in the possible sources as well as the biological properties and uses of vitamin B_2 riboflavin) has stimulated a desire for a more rapid method f assay than the biological rat growth procedure which is enerally followed. At the present time, while there is no fficial biological method of assay, the one of choice is basically hat of Bourquin and Sherman (*1*). This has been modified y different workers from time to time with the intent chiefly f improving upon the supplemental factors and thereby aking it more specific. One of the chief objections to the ethod is the time it requires—at least 28 days—which not nly entails the factor of cost but interferes markedly with the xecution of many problems of practical interest.

In contrast to the rat growth method, two comparatively apid procedures have been used to a limited degree for de-ermining flavin. The first measures the degree of fluores-ence given by flavin in violet light. This can be done with uitable filters using either a visual procedure with the Fluo-ay or similar type of instrument as described by Supplee and thers (*7*), or by photoelectric fluorescence measurement as eveloped by Cohen (*2*), and Euler (*3*) and adapted by Hand *4*). The second method, the microbiological procedure of nell and Strong (*6*), measures the influence of flavin on oth the cell growth and the acid production of *Lactobacillus asei* ϵ grown on a synthetic medium free of flavin.

The present study is a report of progress with these meth-ds as applied concurrently to several samples varying in ype, composition, and potency.

Methods

BIOLOGICAL METHOD. The ration consisted of purified casein 8 per cent, sucrose 68 per cent, Crisco 9 per cent, McCollum alt mixture No. 185 4 per cent, and cod liver oil 1 per cent. This vas supplemented with daily treatment of 16 micrograms of hiamine hydrochloride and 0.08 gram of Labco rice polish extract reed from flavin with fuller's earth. The rats at the start were 2 to 25 days of age and weighed 40 to 50 grams. The depletion eriod ranged from 2 to 3 weeks. The test period was 4 weeks. Concurrently with the unknown samples, fed at different levels, groups of rats were treated daily with 2.5 , 3.75, and 5.0 micro-rams of riboflavin, respectively. These groups served as stand-rd controls and permitted one to calculate the potency in terms f riboflavin.

FLUORESCENCE METHODS. (*a*) In cases where the riboflavin night have been in partial combination, the sample was digested s follows: five grams were suspended in 100 cc. of water, about .1 gram each of papain and of standard takadiastase were added, nd the mixture was digested at 37° to 40° C. for 2 hours. Then .2 cc. of glacial acetic acid was added. The whole was heated o 80° C. to coagulate the protein and then filtered or centrifuged. The readings were made on the clear solution.

(*b*) If the preparation was too dark, decolorizing was carried ut by the method of Kemmerer (*5*) which is a modification of hat of Van Eekelen and Emmerie (*8*). This consists of making a nethanol extract, adding a few cubic centimeters of glacial acetic cid, filtering if necessary, adding a few drops of saturated solu-ion potassium permanganate, and then a little fresh hydrogen eroxide. The fluorescence was measured on this decolorized inal solution.

In some cases it was necessary to introduce adsorption and lution steps, making the determination of the riboflavin on the luate.

(*c*) For the visual reading the Fluoray Eveready carbon lamp vas employed, fitted with a red-purple Corning filter No. 587 nd a Corning filter No. 351 to screen out interfering blue luorescence.

In assaying an unknown, at least three dilutions were made, preferably in the range of 0.1 to 0.2 microgram of riboflavin per cc. These were read against suitable standards and the potency was computed from the average values.

(*d*) The photoelectric fluorescence measurements were made with the Pfaltz & Bauer fluorophotometer. In this instrument emission from a mercury capillary arc is filtered (Corning glasses 038 and 511) and directed through a solution of the riboflavin. The fluorescent light strikes a photocell at the side of the cuvette and is transformed into electrical energy, the current of which is measured by a galvanometer. This photocell is protected from the incident intensity by Zeiss filter No. OG1. With a constant incident intensity, there is a linear relationship between the deflection of the galvanometer and the concentration of the riboflavin.

In preparation of the sample, a 10-cc. portion of the riboflavin solution was diluted with twice the volume (20 cc.) of acetone as suggested by Hand (*4*). The solution was agitated for a few seconds. If a precipitate formed the solution was filtered, the first part of the filtrate being passed again through the filter.

After suitable dilution, the fluorescence was then read directly in the cuvette, and the concentration of the riboflavin determined by reference to a standard curve prepared from dilutions of pure riboflavin.

MICROBIOLOGICAL METHOD. This procedure was carried out according to the method of Snell and Strong (*6*). The growth response of the bacteria was measured in terms of culture turbidity expressed as galvanometer extinction by means of the Pfaltz & Bauer fluorophotometer; and acidimetry, measuring the amount of acid produced in 72 hours' incubation. In this connection an innovation was introduced. The cultures were grown in carefully standardized 16 × 150 mm. test tubes which could be inserted directly into the light path of the fluorophotometer for extinction measurements at the end of 21 to 24 hours' incubation. Following this, the cultures were returned to the incubator to complete a 72-hour incubation, when the contents of each tube were removed and titrated against standard alkali. In this way both determinations were made on one and the same culture tube.

Samples were prepared for assay by simple water dilution except in the case of dry yeasts. These were digested with the enzymes as outlined above. The standard riboflavin solution was run for each assay in duplicate at five levels ranging from 0.05 to 0.15 microgram per 10-cc. culture tube. The unknowns were run in groups of 5 tubes covering this same range of dilutions. Response curves for the standard riboflavin were constructed from the turbidity values and from the acidimetric values. The unknowns were evaluated in terms of these and expressed as micrograms of flavin per gram.

Discussion

In Table I the results are given as micrograms of riboflavin per gram of sample for the four procedures—biological rat growth, microbiological, fluorophotometric, and Fluoray. The type of samples begins at the top with relatively low-potency extracts of grain which are rich in sugars, followed by samples of dried yeast and extracts of the same; by liver extracts, plain and fortified with other vitamins; by high concentrates; and finally by blends of the more potent types with the low ones, high in sugar.

Observing these data for the various groups, the respective assay values for the four methods show a general tendency toward agreement. The largest differences occur, as one might expect, in the samples that are lowest in potency. This is brought out by comparing the values by the respective methods with the average of data by all the methods, given in the last column. If the average deviations from the mean are calculated for the different methods, that for the biological

Table I. Comparative Potencies, Determined by Different Methods

(Expressed in micrograms of riboflavin per gram)

Sample Number	Description	Biological	Microbiological	Fluorophotometric	Fluoray	Average of All Methods
38,540	Wheat germ extract	4.5	3.2	2.7	3.7	3.5
40,480	Rice polish extract	9	7.1	10.9	6.4	8.4
39,590	Rice bran extract	10	8.5	9	6.5	8.5
35,120	Yeast, dried	62	46	51	65	56
36,950	Yeast, dried	57	50.5	54	48	52.4
34,900	Yeast concentrate	200	171	192	195	189.5
38,790	Liver extract, dried	208	195	246	230	220
33,509	Liver extract, dried	230	204	234	225	223
39,140	Liver extract, dried	285	207	226	300	254.5
33,139	Liver extract, dried	500	501	507	537	511
20,879	Liver extract, fortified	250	277	302	244	268
23,659	Liver extract, fortified	250	241	269	216	244
40,360	Liver extract, fortified	682	670	685	727	691
34,880	Special concentrate	4690	4610	4800	4900	4750
37,630	Special concentrate	4685	5260	5470	4680	5024
40,160	Special concentrate with wheat germ	942	940	965	934	945
40,170	Special concentrate with wheat germ	317	312	347	340	329
40,420	Yeast concentrate with wheat germ	102	88	85	89	91
40,430	Yeast concentrate with wheat germ	135	114	109	134	123

assay is lowest, about 8 per cent. The other three are very similar, 10 to 12 per cent from the mean.

In Table II the microbiological, fluorophotometric, and Fluoray results in Table I are expressed as per cent difference (plus or minus) from the corresponding biological values. While the biological rat growth method is generally considered the standard criterion, such results in themselves are not necessarily correct to within 10 to 15 per cent. Nevertheless it is of interest to make the comparison. It is seen that there are some rather wide variations which in general occur with the low-potency samples. The averages of the per cent differences (including sign) are, respectively, −10.7, +5.3, and −7.0 by the microbiological, fluorophotometric, and Fluoray methods.

The question then arises as to what advantage, if any, one of these three methods has over the other, granting that they all require much less time than the biological method. In general, with the fluorometric measurement, it is often difficult to know how and when to treat the samples so as to obtain the optimum value. As a result, there is entailed considerable preliminary experimentation by trial and error. In addition, with the visual (Fluoray) procedure there is apt to be a large personal error unless the operator has had much experience. With the photoelectric procedure, there are also objections due to possible solarizing or cell fatigue. This entails a frequent careful checkup and restandardization of the instrument before each series of runs.

Since the microbiological method of Snell and Strong seemed to possess certain advantages, it was tested out further as to comparison of results obtained by turbidity extinction with those by acidimetry, reproducibility, and specificity in relation to the influence of other water-soluble vitamins.

The data for the comparison of extinction and acidimetric determinations are given in Table III for the same samples that were reported on in Tables I and II. As stated previously, the extinction values represent the culture turbidity formed in 24 hours' incubation and the acidimetric values represent acidity produced during 72 hours' incubation. The two sets of data show remarkable agreement throughout, irrespective of type or degree of potency of the samples. This fact is brought out clearly in the last column, giving the ratio of the two corresponding values, the average (19 samples) being 0.981. This shows that under the conditions as outlined either procedure gives the same end results.

The reproducibility of the method was tried on a liver extract (sample 39,140). In all, fifteen separate runs were made. The data are given in Table IV and express extinction values. The micrograms of riboflavin per gram (column 2) ran very close, averaging 207, with an over-all range of 8.3 per cent and an average difference from the mean of only 2.

Table II. Deviation from Bioassay Values

Sample Number	Description	Bioassay	Difference from Bioassay Value: Microbiological	Fluorophotometer	Fluoray
		μg./g.	%	%	%
38,540	Wheat germ extract	4.5	−28.9	−40.0	−17.
40,480	Rice polish extract	9.0	−21.2	+21.1	−28.
39,590	Rice bran extract	10	−15.0	−10.0	−35.
35,120	Yeast, dried	62	−25.8	−17.8	+ 4.
36,950	Yeast, dried	57	−11.4	− 5.3	−15.
34,900	Yeast concentrate	200	−14.5	− 4.0	− 2.
38,790	Liver extract, dried	208	− 6.3	+18.0	+10.
33,509	Liver extract, dried	230	−11.2	+ 1.5	− 2.
39,140	Liver extract, dried	285	−29.8	−20.7	+ 5.
33,130	Liver extract, dried	500	+ 0.2	+ 1.4	+ 7.
20,879	Liver extract, fortified	250	+10.8	+20.8	− 2.
23,659	Liver extract, fortified	250	− 3.6	+ 6.5	−13.
40,360	Liver extract, fortified	682	− 1.8	+ 0.5	+ 6.
34,880	Special concentrate	4690	− 1.9	+ 2.4	+ 4.
37,630	Special concentrate	4685	+12.3	+16.7	− 0.
40,160	Special concentrate with wheat germ	942	− 0.3	+ 2.4	− 0.
40,170	Special concentrate with wheat germ	317	− 1.6	+ 9.4	+ 7.
40,420	Yeast concentrate with wheat germ	102	−13.8	−16.7	−12.
40,430	Yeast concentrate with wheat germ	135	−15.6	−19.9	− 0.
	Average of 19, including sign		−10.7	+ 5.3	− 7.

per cent. These values indicate that the method gives good reproducibility.

In respect to specificity, Snell and Strong (*6*) reported that such substances as copper, manganese, iron, zinc, barium, pyridine, and alcohol did not inhibit the growth of the *Lactobacillus casei*. The question arose as to the specificity of the organism for riboflavin in the presence of the other water-soluble vitamins—that is, whether they might act as accelerators or inhibitors. The findings are given in Table

Table III. Comparison of Extinction and Acidimetric Results of Microbiological Method

Sample Number	Description	Extinction, I	Acidimetry, II	Ratio II/I
		Micrograms per gram		
38,540	Wheat germ extract	3.2	3.3	1.03
40,480	Rice polish extract	7.1	7.1	1.00
39,590	Rice bran extract	8.5	8.2	0.96
35,120	Yeast, dried	46	44	0.96
36,950	Yeast, dried	50.5	50	0.99
34,900	Yeast concentrate	171	162	0.95
38,790	Liver extract, dried	195	194	1.00
33,509	Liver extract, dried	204	183	0.90
39,140	Liver extract, dried	207	210	1.02
33,139	Liver extract, dried	501	431	0.86
20,879	Liver extract, fortified	277	275	0.99
23,659	Liver extract, fortified	241	239	0.99
40,360	Liver extract, fortified	670	673	1.00
34,880	Special concentrate	4610	4520	0.98
37,630	Special concentrate	5260	5270	1.00
40,160	Special concentrate with wheat germ	940	954	1.02
40,170	Special concentrate with wheat germ	312	310	0.99
40,420	Yeast concentrate with wheat germ	88	90	1.02
40,430	Yeast concentrate with wheat germ	114	113	0.99
			Av.	0.98

ABLE IV. REPRODUCIBILITY OF MICROBIOLOGICAL METHOD FOR RIBOFLAVIN

(Liver extract 39,140)

Test	Micrograms per Gram	Difference from Mean	Per Cent Difference
1	207	0	0.0
2	212	+5	2.4
3	215	+8	3.9
4	206	−1	0.5
5	209	+2	1.0
6	213	+6	2.9
7	200	−7	3.4
8	214	+7	3.0
9	207	0	0.0
10	198	−9	4.4
11	199	−8	3.9
12	207	0	0.0
13	206	−1	0.5
14	211	+4	1.9
15	202	−5	2.4
	Av. (15) 207		2.5

/ as galvanometer extinctions for 0.1 microgram of riboflavin per 10-cc. tube, first alone as control, and successively when combined with varying concentrations of thiamine, pyridoxine, nicotinic acid, pantothenic acid, ascorbic acid, and finally a combination of all six vitamins. The values represent extinction readings for individual tubes. Since they do not vary in any case more than ±10 per cent from the readings for the tubes containing riboflavin alone, it is evident that the method measured only riboflavin and that the supplemental vitamins had no stimulating or retarding effect.

Summary

A comparison has been made of four methods for determining vitamin B_2 (riboflavin): biological rat growth; visual fluorescence; photoelectric fluorescence; and microbiological, by both culture turbidity and acidimetry. The four methods gave similar results; the greatest differences were with the low-potency samples.

In a further study the microbiological method showed excellent specificity and reproducibility. The results were almost identical whether measured as culture turbidity at the end of 24 hours' or acidimetry at the end of 72 hours' incubation.

TABLE V. SPECIFICITY OF MICROBIOLOGICAL METHOD FOR RIBOFLAVIN

(Influence of other vitamins on galvanometer extinction)

Added per 10-Cc. Tube	0.1 μg. of Riboflavin Alone	Factor Added: Thiamine	Pyridoxine	Nicotinic acid	Pantothenic acid	Ascorbic acid	All combined
μg.							
0.1	0.52	0.55	0.53	0.52	0.54	0.54	0.53
1	0.52	0.55	0.51	0.53	0.51	0.52	0.50
10	0.53	0.54	0.53	0.51	0.53	0.49	0.53
100	0.52	0.53	0.53	0.53	0.53	0.54	0.50
1000	0.53	0.53	0.52	0.53	0.52	0.57	0.56

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Chemical Estimation of Nicotinic Acid and Vitamin B_6

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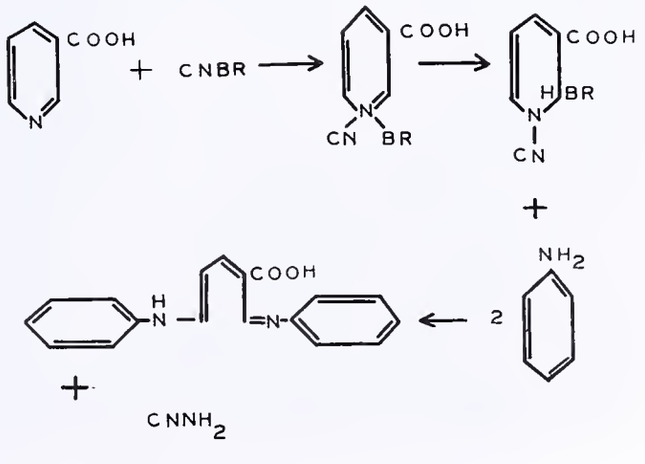
THE chemical methods now available for the determination of nicotinic acid depend upon the pyridine ring structure. It is well known that the pyridine ring is more reactive than the benzene ring and this greater reactivity is due to the nitrogen in the pyridine ring. The proposed methods depend on the breakdown of the pyridine nucleus with the liberation of nitrogen, thus opening the ring, followed by conjugation of the carbon chain with some aromatic amine.

A number of compounds have been used to react with the tertiary nitrogen for the purpose of splitting the pyridine ring. Vongerichten (*22*) and Reitzenstein (*15*) first proposed 2,4-dinitrochlorobenzene as the compound to add to the nitrogen. König (*9*) used cyanogen bromide, phosphorus trichloride, and other compounds. Once the nitrogen has its greatest valence satisfied by the addition of any one of these compounds, the ring is more susceptible to decomposition. The ring is easily broken with the liberation of the nitrogen in the form of a tertiary amine, thus yielding the carbon skeleton that combines with the amine to give a stable colored compound. The skeleton of the ring is a derivative of glutaconic aldehyde, which readily enolizes to give a reactive hydroxyl which can then combine with the organic base. The di-anil reaction illustrated



is with cyanogen bromide, but dinitrochlorobenzene and phosphorus trichloride will also give this reaction.

The aromatic amine plays an important role in the development of the color. Aniline, the simplest aromatic amine, was first used with cyanogen bromide, while later other organic bases were used which included β-naphthylamine, sulfanilic acid, *p*-phenetidin, cumidene, xylidine, etc. Bandier and Hald (*3*) used sodium sulfanilate, *p*-aminophenol, and sulfanilamide and found that none of these gave as consistent results as did *p*-methylaminophenol under the conditions employed. The authors have used aniline with satisfactory results. Shaw and McDonald (*19*), Melnick and Field (*12*), Pearson (*13*), and Swaminathan (*20*) used this amine to advantage. von Euler *et al.* (*4*) claimed better results with β-naphthylamine. Harris and Raymond (*6*) and Kodicek (*8*) used *p*-amino acetophenone and Arnold, Schreffler, and Lipsius (*1*) obtained good results with this amine.

Specificity

A most important consideration in any colorimetric method is the specificity of the reaction. Natural materials often contain closely related compounds which give similar chemical reaction but dissimilar biological activity. One known vitamin other than nicotinic acid that contains the pyridine ring is vitamin B_6. This substance does not give the cyanogen bromide color reaction. It has been stated by some workers that the cyanogen bromide reaction is specific for pyridine compounds having the trivalent nitrogen. This is erroneous, since good evidence supports the theory that the alpha position must be unsubstituted for the complete cyanogen bromide color development. Since vitamin B_6 has a methyl group in the alpha position, it is to be expected that no color will develop. Bandier (*2*, *3*) found that if the analysis is carried out with *p*-aminophenol sulfate in the presence of potassium dihydrogen phosphate, small amounts of pyridine will not give the color. Picolinic acid, α-picoline, trigonelline, and methylpyridinium chloride do not give the color under the conditions stated.

A great number of compounds have been tested by the cyanogen bromide reaction, and many investigators have found that all the amino acids, a variety of purines, pyrimidines, certain metabolites, and carbohydrates will not give the yellow color. The extensive list indicates that a great many chemically important cell constituents are inactive in this test. However, compounds such as β-picoline, α-aminopyridine, and nepecotic acid have given positive tests. α-Aminopyridine probably gives the test since the amino group enters into the di-anil reaction. It is fortunate that these compounds must be present in high amounts to give a fair color, and more important still is the fact that these compounds probably occur in very small amounts if at all in biological materials.

Although the reaction is highly specific, certain pieces of evidence emphasize the limitations of the method. From the data obtained independently in the authors' laboratory (*23*) and by Kodicek (*8*), it is apparent that certain plant materials give values for nicotinic acid which are not reconcilable with the known fact that cereals are low in the anti-black-tongue or antipellagra factor. For example, the corn which the authors used for analysis was used in the routine production of black tongue in dogs, yet the high nicotinic acid value obtained by analysis of this corn was an indication that certain unknown substances do give the color reaction and yet have no antipellagra activity. Karrer and Keller (*7*) have stated the possibility that plant materials may contain pyridine type alkaloids which would account for the apparent high values for nicotinic acid. The authors have found very high color development in samples of polished rice, wheat middlings, wheat germ, whole wheat, and various cereal brans. It appears that the chromogen responsible for the color is tied up and is liberated only on hydrolysis. Kodicek (*8*) and Waisman, Henderson, and Elvehjem (*23*) have found that a water extract of corn will remove the nicotinic acid but leave the accompanying chromogen behind. The values obtained by this extraction procedure are more in keeping with the known facts.

Conditions of Reaction

Colored Complex. The colored derivative formed from nicotinic acid, cyanogen bromide, and aniline is the simplest reaction product. It is soluble in water, but as amines of higher molecular weight are used the solubility of the colored complex decreases. Various workers have attempted to extract the colored complex into suitable solvents in order to stabilize the color by removing it from the excess reagents. Swaminathan (*20*) used amyl alcohol, Ritsert (*16*) and Perlzweig, Levy, and Sarett (*14*) used butanol, but in the authors' laboratory (*23*) these solvents proved unsatisfactory for color comparison. Arnold and co-workers (*1*) employed ethyl acetate for extraction of the color formed when *p*-amino acetophenone was used.

Absorption Maxima. In general, the majority of investigators have used photometers, tintometers, or photoelectric colorimeters for reading the color developed. The readings are made with filters that cut out all wave lengths other than those between 420 and 440 millimicrons. There have been reported slight variations from this maxima. Perlzweig and co-workers (*14*) claim that the true absorption maximum lies at 400 millimicrons. The authors have found that reliable readings are obtained with 400, 420, or 440 millimicron filters.

Time of Reaction. The time at which the colorimetric readings are made varies, depending upon the particular amine used and the conditions under which the color is developed. Various workers have used widely differing procedures and when aniline is used the readings can be made within 5 minutes. It would seem advisable to plot a time curve for a particular procedure that is to be used by the investigator. When amines other than aniline are used the color is developed more slowly.

H-Ion Concentration. The hydrogen-ion concentration of the test solution has a definite effect on color intensity. A number of workers have adjusted the solution to be tested to pH 7 before the development of color, while others have shown that there is a definite decrease in intensity with a decrease in pH. Using β-naphthylamine, von Euler and coworkers (*4*) found the color intensity to decrease on either side of the optimum pH. From the observations of many workers it seems that the optimum pH range in which there is a nonvarying color maximum is between 5.5 and 7.5.

Influence of Salts on Color. The detrimental influence of some salts on the development of color has been a difficulty to most workers who have attempted to perfect a chemical method for the determination of nicotinic acid. This difficulty has been one reason for extracting the color with organic solvents.

Bandier and Hald (*3*) found sulfate and nitrate ions to cause no change in the tint, but claimed that acetate ions interfered. Sodium chloride, ammonium chloride, and potassium dihydrogen phosphate have no effect on the color. von Euler *et al.* (*4*) found acetates and sulfates to interfere but alkali chlorides had no effect. These two workers used amines other than aniline, which might account for the divergent observation of Kringstad and Naess (*10*) that alkali chlorides in low concentrations gave increased intensities. Kodicek (*8*) found bicarbonate to cause a slight fading, and in contrast to the results of von Euler, found the acetate ion to have no effect on color development, but these two workers used different amines which very likely influenced the results they obtained. The authors have observed that salts in low concentrations affect the color adversely when aniline is used (*23*).

Table I. Nicotinic Acid Content of Biological Materials

Sample	Waisman *et al.*	Melnick and Field	Bandier	Kringstad and Naess	Kodicek	Pearson	Swaminathan
	Milligrams of nicotinic acid per 100 grams of fresh material						
ef liver	20.2	...	12.2	20.0	17.0	...	12.5 (sheep)
rk liver	28.2	...	11.8	...	...	...	...
ef muscle	4.8	...	4.9	4.9	4.3	...	...
rk muscle	5.3	...	4.7	3.3	...	...	...
ef spleen	4.7	...	4.4	...	5.3	...	...
noked ham	8.3	...	...	...	...	...	...
ewer's yeast	...	43.0	53.0	44.5	57.5	...	57.0
ood	...	0.72 (man)	...	...	0.45 (sheep)	0.80 (sheep)	0.4 (man)
w's milk, 100 cc.	0.82	0.45	...	...	...	...	...
e	...	...	1.5 (cod)	1.4 (frog)	2.0 (herring)	...	...
ied egg white	5.2	...	...	...	...	...	...
lished rice	10.5 (?)	...	...	...	...	...	...
ellow corn	10.7 (?) (acid hydrolysis)	...	...	...	4.5 (alkaline hydrolysis)	...	...

Effect of Light on Color. The effect of light on the ability of color is a consideration only when amines of high olecular weight are used. The color is developed more owly and light seems to have a detrimental effect (*3*). With niline the developed color is read within 5 to 7 minutes and his effect is of minor consideration. However, the color evelopment is best carried out in diffuse light as a precaution against a possible detrimental effect of light.

Quantity of Amine. A number of workers have found hat the amount of amine used is important, especially organic amines other than aniline. Bandier and Hald (*3*) ound that the final intensity of the color depends on the etol concentration and not on the absolute amount of metol. he majority of the investigators have used an excess of the mine in the development of the color, but it appears (*10*) hat for a given amount of cyanogen bromide a definite mount of amine should be used.

Quantity of Cyanogen Bromide. The amount of cyanogen bromide used by various investigators has varied from .35 to 6 ml. of the 4 per cent solution. Some workers have ound that for a given amount of nicotinic acid a certain uantity of cyanogen bromide must be used. It appears from nost of the available data that the exact amounts of cyanogen romide used are not too important, but rather that if an xcess is present no difficulties should be encountered in deveoping the maximum color.

Effect of Heat. Kodicek (*8*), Bandier and Hald (*3*), and Arnold and co-workers (*1*) heated the tubes in order to complete the reaction of cyanogen bromide and nicotinic acid. In early work in the authors' laboratory (*23*) they found that this procedure is especially advisable when higher molecular weight amines are used. Although heating the solution will likewise hasten the reaction when aniline is used, the added manipulations involved in the heating and cooling do not compensate by increasing the sensitiveness of the reaction.

Some Difficulties Involved in the Determination

An analysis of biological material for nicotinic acid necessitates the liberation of the vitamin from combined forms such as coenzymes I and II. Many investigators have used either alkali or acid hydrolysis in order to obtain the free nicotinic acid or amide. Acid hydrolysis has the advantage that one obtains the free acid which is best suited for the color development. A number of workers have made water extracts of the material to be investigated followed by acid hydrolysis, thus preventing the interference by the yellow color due to the humin resulting from the hydrolysis of the proteins. Although no work has been published on enzymatic hydrolysis of naturally occurring materials, this procedure remains as a possibility for the liberation of nicotinic acid from complexes not easily broken down by short acid or alkaline hydrolysis.

The occurrence of interfering colors in natural materials such as bile pigments, riboflavin, carotenoids, and perhaps other unknown compounds has proved a definite handicap in reading the color developed by the reagents with the nicotinic acid present in the sample. Various workers thus found it necessary either to remove the nicotinic acid from the interfering pigments or to remove the pigments from the nicotinic acid. In some early work in their laboratory the authors found it desirable to eliminate the interfering yellow color that one obtains in liver extracts and in hydrolyzed biological materials. Attempts were made to remove interfering colors by adsorption on norite, by filtration methods, by extraction of the nicotinic acid into suitable solvents, and by differential solubility, but none of these methods was satisfactory. The use of preferential adsorption of interfering pigments on charcoal has been aided by the Melnick and Field (*12*) modification of the Shaw and McDonald (*19*) procedure of an acid alcohol adsorption with a special charcoal.

Reagents Used

Cyanogen Bromide. A cold saturated bromine water solution is carefully decolorized in the cold with 10 per cent sodium cyanide with the aid of a buret. Care is taken to prevent having any excess of the cyanide. Sodium is preferable to the potassium salt for making the reagent. This 4 per cent solution of the reagent is kept cold when not in use and is stable for long periods.

Alcoholic Aniline Solution. A 4 per cent solution is prepared by dissolving freshly distilled aniline in absolute ethyl alcohol.

Standard Nicotinic Acid Solution. This contains 50 micrograms of nicotinic acid per cc. of absolute ethyl alcohol. A water solution containing 50 micrograms per cc. is also made.

Buffer Solution. This consists of 980 cc. of water, 15 cc. of 15 per cent sodium hydroxide, 5 cc. of 85 per cent phosphoric acid, and 167 cc. of absolute alcohol. This buffer solution corresponds to the pH of the test solution and the reagents.

Procedure

The procedure used is essentially that of Melnick and Field (*12*), with slight modifications to simplify parts of the procedure. A sample containing 25 to 200 micrograms of nicotinic acid is weighed into each of two 15-cc. graduated centrifuge tubes. To one of the tubes are added 50 micrograms of nicotinic acid in water solution, to act as recovery. Five cubic centimeters of concentrated hydrochloric acid (sp. gr. 1.18) are added to both tubes together with 5 cc. of distilled water. The material is placed in a boiling water bath for 30 to 40 minutes with occasional stirring.

After hydrolysis the tubes are cooled, made up to the 15-cc. mark, and transferred to 125-cc. Erlenmeyer flasks. The centrifuge tubes are washed out carefully with exactly 10 cc. of absolute ethyl alcohol. To the flask are added 200 mg. of Darco (a charcoal obtained from the Coleman and Bell Co., Norwood, Ohio). The flasks are shaken, then filtered through quantitative filter paper into dry 50-cc. Erlenmeyer flasks. An 8.33-cc. aliquot of the filtrate is pipetted into a graduated centrifuge tube, 1 drop of phenolphthalein is added, and the solution is adjusted to pH 8.5 in the cold by the dropwise addition of saturated sodium hydroxide. The solution is now adjusted to pH 7.0 with the aid of an outside indicator, bromothymol blue, using dilute hydrochloric acid. Care must be taken in neutralizing so that the final volume is no more than 10 cc. The alcohol-water ratio at this final dilution is 1 to 2.

To carry out the color development so that readings can be made in the Evelyn photoelectric colorimeter, eight colorimeter tubes are used. To the first and second tubes are added 1 cc. of absolute ethyl alcohol and 2 cc. of water; to tubes 3, 4, and 5 are added 3-cc. portions of the solution of the sample; 3-cc. portions of the sample and recovery are added to tubes 6, 7, and 8; 7 cc. of the buffer solution are added to tubes 1, 3, and 6. Tubes 3 and 6 thus furnish the independent blank determination for the residual color of the sample remaining after decolorization with charcoal. Tube 2 acts as the blank on the reagents. To tubes 5 and 8, 5 micrograms of nicotinic acid are added in order to obtain the photometric density of this known amount of nicotinic acid.

To tubes 2, 4, 5, 7, and 8, 6 cc. of cyanogen bromide are added, followed as rapidly as possible by 1 cc. of alcoholic aniline. The tubes are shaken and the colorimetric readings made within 5 to 6 minutes after the addition of the aniline to the last tube. To read the color in the tubes, filter 400 or 420 millimicrons is used. Tube 1 is set so that the galvanometer reads 100. Tubes 3 and 6 are read at this setting. Tube 2 is now set at 100 and the readings for tubes 4, 5, 7, and 8 are made with this setting. In this way the double blank is obtained which checks both the reagents and the residual color in the test solutions.

Calculations of Reading with Evelyn Photoelectric Colorimeter

$$K_1 = \frac{\text{5 micrograms of nicotinic acid}}{\text{log tube 5} - \text{log tube 4}}$$

$$X_1 = K_1 \times (\text{log tube 4} - \text{log tube 3})$$

$$K_2 = \frac{\text{5 micrograms of nicotinic acid}}{\text{log tube 8} - \text{log tube 7}}$$

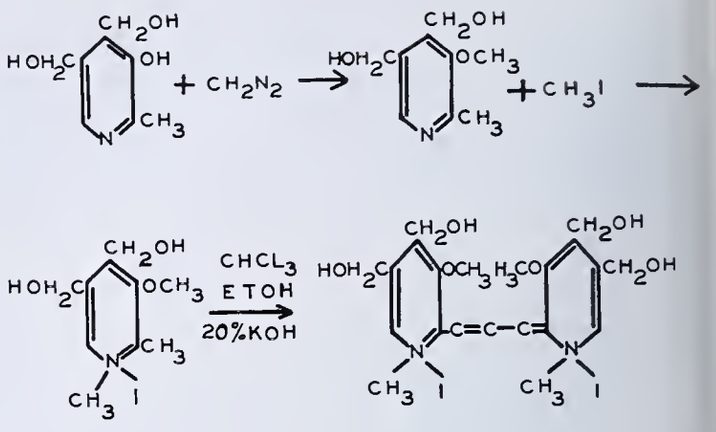
X_2 = $K_2 \times$ (log tube 7 − log tube 6), where X_1 is the nicotinic acid content of the aliquot of the sample

$X_2 - X_1$ should equal 5 micrograms, since the 3-cc. aliquot of the neutralized filtrate represents one tenth of the original sample, and the original recovery sample had 50 micrograms of nicotinic acid added. For more complete details on the method of calculation and procedure reference should be made to Melnick and Field (*12*) and to (*17*).

The methods now available to the investigator leave something to be desired. However, accepting the limitations of the cyanogen bromide method in general, it can be applied with a fair amount of success to the determination of nicotinic acid in animal tissues, urine, and blood. The analysis of plant materials must await further investigation before acceptable values are obtained. For practical purposes, however, the relative amounts of nicotinic acid in a variety of samples are important and the absolute values although desirable are not necessary. A few nicotinic acid values are given in Table I as determined in the authors' laboratory and by other investigators.

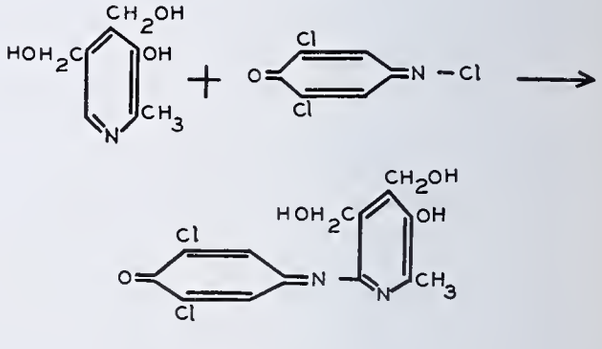
Chemical Estimation of Vitamin B_6

The formula for pyridoxine (vitamin B_6) as now definitely established has the basic pyridine ring with two hydroxy methyl substituents, one alpha methyl group, and a phenolic hydroxyl in the beta position. Kühn and Löw (*11*) have applied the Folin Denis phenol reaction to the vitamin and found the color to be very sensitive. One difficulty which they observed was that of bleaching of the blue color by precipitates that are formed. Kühn and Löw (*11*) also attempted to determine this vitamin by forming carbopyridinium cyanin compounds. This reaction depends on the presence of a methyl group in the alpha position of the pyridine ring. In order to prepare the derivative, the beta-hydroxyl is methylated and the ether formed by diazomethane. Then by using methyl iodide and conversion into the quaternary pyridinium compound the product can be converted to a blue color by slight heating with chloroform and 20 per cent alkali in the presence of alcohol. The reactions are the following:



The carbopyridinium cyanin compound formed from the methyl ether methyl iodide is specific for vitamin B_6. Straight-line curves are obtained with crystalline vitamin, but the obvious difficulties are the quantitative conversion of the vitamin into its various derivatives. However, Kühn and Löw claim that the color developed either with the phenol reagent or with the quaternary pyridinium compound obeys Beer's law. These investigators have given no data on the application of their method to biological materials.

Scudi, Koones, and Keresztesy (*18*) have used a modification of the Gibbs phenol test (*5*), which depends on the formation of indophenols. These are products of the interaction of phenols and a dihalogen substituted quinone chloroimide. An intense blue solution results from the interaction of the phenolic hydroxyl of vitamin B_6 and the chloroimide.



It is believed that only those phenols react which have an open position para to the phenolic hydroxyl. The above-mentioned investigators used a veronal buffer to adjust the pH of the material, urine in this case, to pH 7.6 before the addition of the butanol solution of the chloroimide reagent. Extraneous substances are shaken out and the butanol layer is read after 40 minutes. No report has been made of applying this method to biological materials other than urine.

Swaminathan (*21*) has published a method for the chemical determination of vitamin B_6 which involves the use of diazotized sulfanilic acid. Contrary to the contention of Kühn and Löw that the diazotized sulfanilic acid reaction is unreliable, this investigator has obtained values which compare favorably with bioassays. The chemistry involved in the coupling reaction is simply that of forming an azo dye. The diazotized sulfanilic acid is treated with the vitamin solution or a concentrate of the vitamin and coupling takes place at the unsubstituted position para to the phenolic hydroxyl as indicated by the reactions:

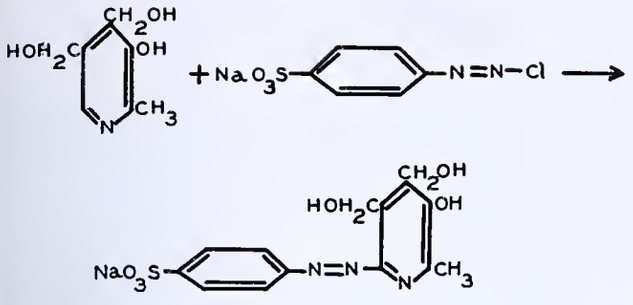


Table II. Vitamin B_6 Content of Foodstuffs

Foodstuff	Henderson, Waisman, and Elvehjem	Swaminathan
Brewer's yeast	40	54
Autoclaved brewer's yeast	40	53
Rice polishings	...	13.4
Sheep liver	12.6	13.4
Sheep muscle	11.7	4.5
Cow's milk	...	1.7
Yellow corn	4.8	7.1
Whole wheat	...	7.4
Polished rice	...	3.0
Soybean	...	8.0
Cabbage	...	3.1
Beet root	...	1.3
Pork liver	9.9	...
Pork ham	24.0	...
Veal muscle	16.3	...
Pork heart	15.5	...

The liberation of vitamin B_6 from naturally occurring materials depends on a pepsin digest followed by tungstate precipitation for removal of proteins and silver nitrate precipitation for the removal of purines, pyrimidines, and amidazole bases. The vitamin was absorbed, then eluted and further treated to remove interfering materials. The pH was adjusted to 7 and the color developed with the diazotized sulfanilic acid. This method is open to criticism since the reaction is not specific. However, the adsorption on an alkaline earth does minimize the carrying over of certain other cell constituents that are claimed to give the color by Kühn and Löw.

Swaminathan has also used the phenol reagent of Folin and Ciocalteau and by the use of either of these two methods, he has obtained the vitamin B_6 content of various foodstuffs. In Table II are listed his values as well as those obtained in the authors' laboratory using a new bioassay procedure for vitamin B_6.

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Chemical Methods for Determination of Vitamin C

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NEARLY all the methods that have been proposed for the estimation of vitamin C have been based upon the reversible oxidation of ascorbic acid to dehydroascorbic acid. Although several of the oxidation methods give reasonably satisfactory results, there are pitfalls to be avoided in all the procedures that have been published; hence it is of distinct value to have available an independent approach, such as that developed by Roe (*9*), in which ascorbic acid is decomposed to furfural for colorimetric estimation. The reaction with 2,6-dichlorophenolindophenol in metaphosphoric acid solution provides the most widely used, and in the author's experience the best, general basis for ascorbic acid analysis. The polarigraphic and oxidation-reduction titrimeter methods are of interest as specialized oxidation procedures, but neither has been developed sufficiently to merit confidence for general use.

Figure 1 will serve to outline the basic steps in the methods that have been proposed. In many types of investigation concerning vitamin C, both in foodstuffs and in physiological research, analyses for dehydroascorbic acid are nearly as important as tests for ascorbic acid itself. This situation arises primarily because dehydroascorbic acid is approximately as active antiscorbutically as ascorbic acid and theories concerning the function of vitamin C *in vivo* are based in part on the analyses for dehydroascorbic acid.

Indophenol Method

For most investigations direct visual titration of ascorbic acid with the dye 2,6-dichlorophenolindophenol in a pH range of 1 to 3.5 gives a reasonably accurate and satisfactory measure of the vitamin C content of tissues. The extracts must be properly prepared and close attention must be given to details of the procedure (*1*, *2*, *7*). The two most crucial points to be watched, apparently, are avoidance of oxidative changes previous to titration, and awareness of the circumstances

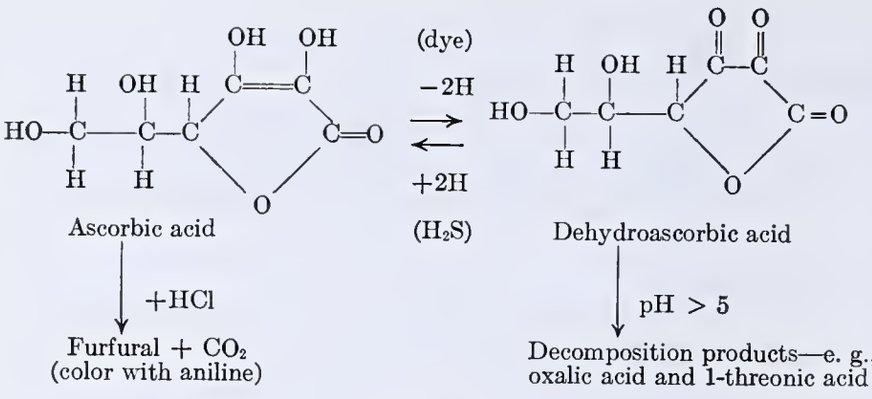

FIGURE 1

under which other reducing substances will or will not interfere with the titration.

In relation to the first point, aerobic oxidation through the agency of enzymes (chiefly copper- but to a lesser extent iron-containing specific proteins) or added copper (picked up from reagents and equipment) can generally be avoided by the use of 2 to 3 per cent metaphosphoric acid as an extractant, if sufficient care is given to other details. Insufficient grinding and extraction of the sample have also been common sources of error. The author has not observed evidence of the existence of significant amounts of combined, non-acid-extracted ascorbic acid as reported by Reedman and McHenry (*8*) and a few other investigators. Neither has he found sulfuric acid, as recommended by Tressler and associates (*5*), to be desirable as an extractant, even though it affords some economy. Although acetic and trichloroacetic acids were widely used in earlier work, and give good results with many products, they are not so satisfactory as metaphosphoric acid. The protective action of acids is not simply a pH phenomenon, because at the same pH, metaphosphoric acid is distinctly superior to sulfuric acid and the latter in turn is superior to hydrochloric acid.

In relation to the second point, sulfhydryl compounds and carbohydrate decomposition products are especially likely to interfere. The concentration of interfering substances in most natural products is not sufficient to cause significant errors, however. When making direct visual titrations it is very important to use an end point of about 5 seconds or less. With the photoelectric colorimeter method, two or more readings should be made at 15-second intervals, from which one can in part correct for the interference due to more slowly reacting substances. Thiosulfate, ferrous salts, and certain sulfhydryl compounds cause interference when present, as do a number of sugar decomposition products having a reductone type of structure.

In some natural products such as strawberries and beets the presence of coloring matter makes it nearly impossible to carry out direct titrations. In such cases the use of a photoelectric colorimeter is satisfactory, however, because one measures only the decrease in color caused by ascorbic acid, when the dye is added, and the natural pigment which masks the disappearance of the indophenol dye is not reduced during the test.

Bessey has described the details of procedure to be followed in using the instrument (*1*) and has cited many related publications. The papers by Mindlin and Butler (*6*) and Farmer and Abt (*3*) are of special interest in relation to blood analyses. It is now generally agreed that cyanide should not be used and that ascorbic acid is fairly stable in whole blood, plasma, and serum except when hemolysis has occurred. There may be a slight loss caused by oxidation arising from ferri forms of compounds and loosely bound oxygen carried by hemoglobin. Bessey has pointed out the value of using an extractant buffered at pH 3.5 for titrations with the instrument, since in that range the reaction with the dye is reasonably specific, the reading does not drift because of a pH effect on the dye, and conditions are about optimum for dehydroascorbic acid analyses. The same author has pointed out the need for a check reading after decolorizing all of the dye, in order to correct for light absorption by suspended matter and other material in the test preparation. In making direct visual titrations one can profitably use a lower pH (about 1) because the slight fading of the dye is then not bothersome and interference by sulfhydryl compounds is markedly decreased.

The indophenol dye that has been used so widely is not ideal, especially because of its relatively high oxidation potential, but it is doubtful whether any other oxidizing agent that has been proposed up to the present time possesses any practical advantage. A few laboratories have reported satisfactory results with methylene blue as an oxidant. The reaction in this case is slow, however, unless there is an accompanying exposure to light. For testing urine samples there have been encouraging reports based upon precipitation of the vitamin by 2,4-dinitrophenylhydrazine, followed by quantitative reduction of the nitro groups or by hydrolysis, reduction, and conversion to furfural. The method proposed by Roe (*9*), based upon furfural formation, has the advantage of completely avoiding interference by non-ascorbic acid reducing material such as sulfhydryl compounds and the 3- or 4-carbon sugar decomposition products, but the procedure is much more time-consuming and necessitates avoidance of possible interfering substances that yield furfural on decomposition, such as the uronic acids and pentoses.

In regard to the use of such special instruments as the oxidation titrimeter or the polarigraph, the author's experience has been very limited, but he has not found that they possess any significant advantage over the photoelectric colorimeter for general use. For meeting special difficulties these instruments have obvious advantages. The polarigraphic method holds promise for detecting and perhaps avoiding errors that arise from interfering reducing substances that exhibit slightly higher or lower reduction potentials, such as characterize the sulfhydryl compounds.

Tests for Dehydroascorbic Acid

In studying ascorbic acid synthesis in tissue slices, Dr. Smythe in this laboratory observed that certain added substrates gave reactions with hydrogen sulfide that were comparable to the reaction generally used for estimating dehydroascorbic acid. The study was extended to establish the fact that many organic compounds give such a reaction. It is reasonably clear that many of the published data relative to the oxidized form of the vitamin have been misinterpreted, because of this interference by newly formed reducing material that results from hydrogen sulfide treatment. The risk of encountering such an error was pointed out in an earlier publication (*4*). The error that is introduced by hydrogen sulfide treatment is also inherent in the modified methods in which mercuric salts are added to remove sulfhydryl compounds from tissue extracts, because these methods include a final treatment with hydrogen sulfide.

Table I lists some of the compounds that were tested and found to interfere seriously with the dehydroascorbic acid determination, together with a number of compounds that did not cause serious interference under the conditions studied. In several cases the interference was almost mole-for-mole equivalent to the reversibly oxidized vitamin. During a considerable part of the titration, the reaction of the newly formed sulfhydryl compounds with the dye was apparently complete within 1 to 5 seconds. Toward the end of the titration there was generally a slower fading of the dye that resembled the typical glutathione reaction. Because of the rapidity of the reaction the interference could not be wholly avoided either by a correction curve with the photoelectric colorimeter or by visual titration at a pH of 1 or less. The best direct methods for detecting and avoiding such an error would seem to be the use of the furfural reaction, the use of a

polarigraph, or to decompose the dehydroascorbic acid at a pH above 7 and note the resultant change in value after hydrogen sulfide reduction.

TABLE I. DEHYDROASCORBIC ACID TESTS

(H_2S for 2 hours at pH 3.5; titration with 2,6-dichlorophenolindophenol)

Positive (Interference > 20%)	Negative (Little or No Interference)	
Pyruvic acid	Acetone	Piperitone
Pyruvic aldehyde	Sorbose	Phorone
Glyceric aldehyde	Levulose	Oxalic acid
Dihydroxyacetone	Glucose	Kojic acid
Acetaldehyde	Xylose	β-Ketobutyric acid
Mannosaccharic acid	Cyclohexanone	β-Ketoglutaric acid
5-Ketogluconic acid	Hexadienal	Glucuronic acid
1,4-Benzoquinone	Butyraldehyde	
1,4-Naphthoquinone		
2-Methylquinone (vitamin K)		

Summary

When adequate precautions are taken, the reaction of ascorbic acid with 2,6-dichlorophenolindophenol can be used in direct titrations or with the photoelectric colorimeter to give relatively satisfactory quantitative analyses. Other methods of analysis may be preferable under special circumstances because of interference by other reducing materials.

Methods for the measurement of dehydroascorbic acid, however, are subject to great interference, because many aldehydes, ketones, and quinones give rise to an interfering reaction when reduced by hydrogen sulfide. Among the interfering compounds are pyruvic acid, pyruvic aldehyde, glyceric aldehyde, dihydroxyacetone, acetaldehyde, mannosaccharic acid, 5-ketogluconic acid, 1,4-benzoquinone, 1,4-naphthoquinone, and 2-methyl-1,4-naphthoquinone (thyloquinone). Three methods for detecting and avoiding such interference are pointed out.

Acknowledgment

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CONTRIBUTION 418 from the Department of Chemistry, University of Pittsburgh.

A Spectroscopic Method for the Quantitative Estimation of Vitamin D

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DURING the past 4 years three independent physicochemical methods (*1*, *5*, *6*, *15*, *16*) have been proposed for the estimation of vitamin D.

The method originally used by Brockmann and Chen has been more extensively studied and modified by several investigators (*3*, *8*, *12*, *13*, *14*). This method is based on the discovery that vitamin D forms with a saturated solution of antimony trichloride in chloroform a pink color which exhibits a prominent band in the region of 500 mμ. Figure 1 shows the antimony trichloride transmission spectra of vitamins D_2 and D_3 and that of the glucoside of vitamin D_2. Irrespective of the minor differences in structure, all transmit exactly in the same region with the head of the characteristic band located at 500 mμ. In the course of their work on the separation of vitamins A and D from fish liver oils (*10*, *11*), the authors followed the purification by measuring the extinction coefficients at 500 and at 620 mμ, the two maxima attributable to vitamins D and A, respectively. By the use of the appropriate correction factors, it was found possible to estimate vitamin D in the presence of considerable quantities of vitamin A with a reasonable degree of accuracy. This method has now been extended and modified to make possible the estimation of vitamin D in fish liver oils.

In preliminary attempts, the authors used as a standard a fish liver oil, the biological potency of which had been accurately determined a number of times by several laboratories and reported to be 50,000 U. S. P. vitamin D units per gram. This oil was kindly supplied by the Atlantic Coast Fisheries Corporation of New York. Using this standard, the potencies of some of the oils tested agreed fairly well with the reported biological potencies. However, a correction for the presence of vitamin A did not improve the spectroscopic values appreciably, since the presence in the oils of sterols and possibly carotenoids gives rise to colors which seem to have an additional effect on the 500 mμ maximum of vitamin D. This made necessary the use of an additional correction for the presence of sterols. Although such a procedure gave satisfactory results for oils containing more than 10,000 U. S. P. vitamin D units per gram, it was found cumbersome and not universally applicable, as it is necessary to have a method of measuring the visible spectrum accurately and rapidly.

In view of these objections the authors have adopted a modified procedure in which vitamin A, 7-dehydrocholesterol,

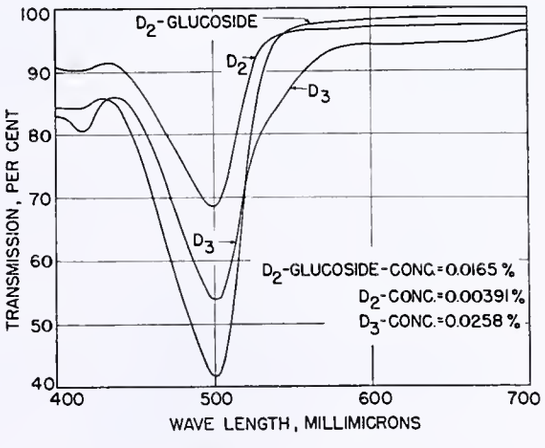

FIGURE 1. TRANSMISSION SPECTRA OF ANTIMONY TRICHLORIDE COLOR OF VITAMINS D_2 AND D_3 AND VITAMIN D_2–GLUCOSIDE

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Table I. Correction Due to Antimony Trichloride

Maximum	Correction
mμ	
430	0.006
455	0.006
480	0.005
500	0.004
580	0.002
620	0.000
690	0.000

and interfering carotenoids are destroyed by a preliminary treatment with maleic anhydride. By the application of this procedure, they obtained satisfactory estimates of vitamin D potency of oils containing in the neighborhood of 10,000 U. S. P. units per gram or higher. In all this latter work, they used as their standard purified crystalline calciferol which had been carefully assayed biologically by R. S. Harris of the Department of Biology. He reported a potency of 40,000,000 U. S. P. vitamin D units per gram. This calciferol was found to have an $E^{1\%}_{1\ cm.}$ value of 890 at 500 mμ (Figure 1, curve II). This modified procedure need not rely for its application upon the use of a rapid means of determining the visible spectrum such as the Hardy color analyzer. Filters suitable for the isolation of a band in the neighborhood of 500 mμ may be used in conjuction with an ordinary colorimeter for the estimation of color intensity. Furthermore, if the sterols are also removed by a preliminary digitonin treatment, it may be possible to estimate vitamin D potencies by measuring the absorption at 265 mμ, and thus avoid the use of antimony trichloride.

General Procedure

To 0.2 cc. of a solution of vitamin D containing oil in chloroform in a 1-cm. special spectroscopic cell are added 3.8 cc. of a saturated solution of antimony trichloride in freshly purified chloroform. The transmission curve (or absorption curve) in the visible region of the spectrum is then determined in the Hardy color analyzer (7) in such a way that the maximum at 620 mμ is reached exactly 3 minutes after mixing. A second curve is then run, so that the maximum at about 500 to 520 mμ is reached 10 minutes after mixing. Finally a third curve is run 30 minutes after mixing.

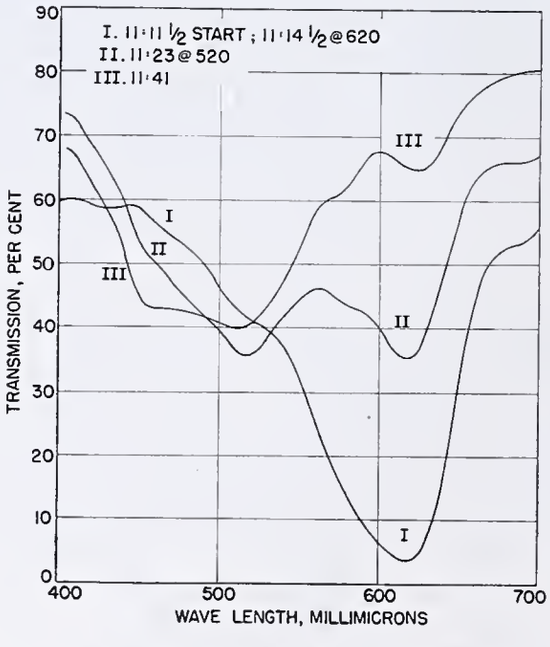

Figure 2. Effect of Time on Transmission Spectrum of Antimony Trichloride Color of a Fish Liver Oil

This gives a family of curves as shown in Figure 2. From the first curve the concentration of vitamin A is easily estimated by computing the extinction coefficient of a 1 per cent solution in a 1-cm. cell at 620 mμ, using the formula $E^{1\%}_{1\ cm.} = 1/c \times \log 1/T$, where c is the per cent concentration and T the per cent transmission as read from the curve. A small correction may be applied for the absorption due to antimony trichloride by adding to the per cent transmission the values given in Table I for the maxima of prominent bands which have been identified with the oils studied. The corrected $E^{1\%}_{1\ cm.} = E$ corrected/c.

The band at about 620 mμ appears most strongly in the first of each group of curves and fades very rapidly with time. It may be seen from Table II that the $E^{1\%}_{1\ cm.}$ values of five different oils at 620 mμ compare very favorably with the values of the same oils obtained at 328 mμ.

Table II. Comparison of $E^{1\%}_{1\ cm.}$ Values of Vitamin A at 328 and 620 mμ

Oil Sample	$E^{1\%}_{1\ cm.}$ (328)	$E^{1\%}_{1\ cm.}$ (620)	$E^{1\%}_{1\ cm.}$ (328)/$E^{1\%}_{1\ cm.}$ (620)
1616	51.1	96	0.5323
1617	32.8	57.5	0.5704
1618	39.7	75.5	0.5258
1619	32.9	60.7	0.5420
1620	43.6	81.5	0.5349

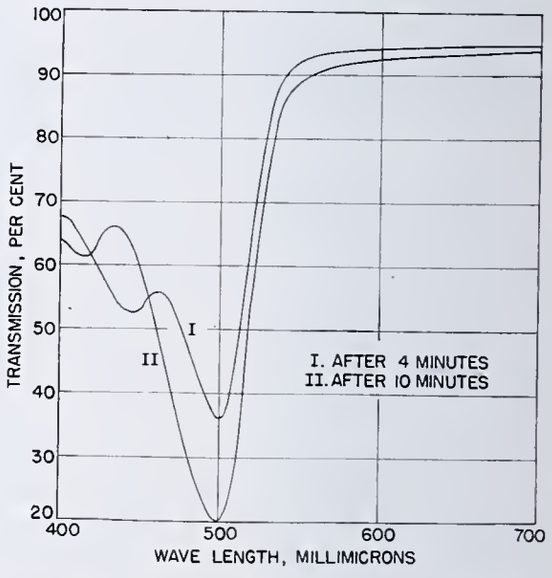

Figure 3. Effect of Time on Transmission Spectrum of Antimony Trichloride Color of Vitamin D_2

The band at 500 to 520 mμ attains a maximum value only after the lapse of about 10 minutes, irrespective of the material containing vitamin D. The effect of time on the development of this band is better illustrated by Figure 3.

A fairly complete spectroscopic study was then made of fifteen fish liver oils which had been assayed biologically, some of them by several laboratories. Six of these oils, marked W_1, W_2, etc., were supplied by C. L. Barthen of the White Laboratories, while the remaining nine were supplied by the Atlantic Coast Fisheries Corporation. All of the important bands in the visible region were accurately located and the extinction coefficient, $E^{1\%}_{1\ cm.}$ for each was estimated. These are recorded in Table III together with the biological potencies and the concentrations of solutions employed. At the bottom of Table III are appended for comparison the

TABLE III. SUMMARY OF SPECTROSCOPIC DATA OF FISH LIVER OILS

Sample	Biological Potency	Concentration	Time	Band$_3$	$E^{1\%}_{1\text{ cm.}}$	Band$_7$	$E^{1\%}_{1\text{ cm.}}$	Band$_8$	$E^{1\%}_{1\text{ cm.}}$	Band$_5$	$E^{1\%}_{1\text{ cm.}}$	Band$_4$	$E^{1\%}_{1\text{ cm.}}$	Band$_3$	$E^{1\%}_{1\text{ cm.}}$	Band$_2$	$E^{1\%}_{1\text{ cm.}}$	Band$_1$	$E^{1\%}_{1\text{ cm.}}$
	U. S. P. units/g.	%	*Min.*																
ACF-CR 503	50,000	0.025	0	430	9.10			472	10.49	520	15.20					620	57.2	690	10.96
			11½			455	11.18			518	17.77	545	14.26	580	14.49	620	18.16	690	7.50
			30			455	14.38	480	14.70	512	15.83			580	8.54	627	7.49	690	3.92
ACF-CR 726	18,000	0.05	0	430	2.72			480	3.76	520	5.12					616	14.10	690	4.60
			10					474	6.26	513	6.16			580	5.25	613	5.60	690	3.20
			30					474	5.90	500	5.52			580	3.38	620	2.92		
ACF 1614	50–100	0.4	0	430	0.286			474	0.42	525	0.70					604	1.49		
			10					468	0.82	525	0.70					600	0.66		
			30					468	0.86	520	0.78								
ACF 1615	50–100	0.2	0	430	0.835			472	1.02	520	1.52			580	1.41	612	4.27	690	0.61
			10					470	1.75	520	1.52			570	0.945	605	1.46		
			30					468	1.96	520	1.46					612	0.79		
ACF 1616	10,000	0.02	0	430	13.65			476	16.67	520	26.3			580	27.95	620	96.0	693	15.35
			10					476	26.20	520	32.7	545	28.5	580	22.70	620	37.85	690	11.10
			16					476	27.50	520	32.1			580		622	29.25	690	10.00
			30					470	27.60	520	28.6			570	18.5	623	21.30		
ACF 1617	30,000	0.02	0	430	9.5			475	11.9	520	16.0					620	57.5	690	11.2
			10					475	15.2	518	18.3			584	14.95	620	18.85	690	7.1
			30					455–75	14.35	510	14.45			570	8.00	620	9.1		
ACF 1618	42,500	0.01	0	430	8.6			455	15.2	518	17.7			580	15.7	618	75.5	690	13.6
			10					455	15.2	512	17.1			580	8.1	622	20.8	690	7.4
			30													624	9.9		
ACF 1619	50,000	0.01	0	430	5.3			455–80	12.6	518	13.4			580	10.7	619	60.7	684	9.8
			10					455–80	11.6	516	12.6			570	5.3	623	14.9		
			30													623	6.8		
ACF 1620	75,000	0.01	0	430	10.4					520	20.5					617	81.5	693	14.8
			10	420	11.8	455	17.8			518	28.4			580	20.6	617	26.1		
			33	420	15.5	455	22.4			510	20.5			580	12.4	617	11.2		
W_1 D-598	275	0.2	0	430	0.345			480	0.39	520	0.490					606	0.96		
			10	430				470	0.54	520	0.490					602	0.45		
			30	430	0.62			460	0.65	520	0.605								
W_2 D-599	800	0.1	0					476	0.80	526	1.04					608	1.86		
			10	420	1.36			476	1.41	526	1.36					608	1.50		
			30					476	1.38	526	1.27								
W_3 D-600	1,500	0.6	0					472	1.52	520	1.40					620	4.2		
			10					468	1.79	520	1.31	545	1.21	580	1.06	620	1.31		
			30	430	0.81			468	1.95	526	1.11			572	0.66	620	0.77	690	0.58
W_4 D-601	3,500	0.05	0					460	0.86	510	1.24					614	3.06		
			11					470	1.76	516	1.76			580	1.32	610	1.32		
			30					460	1.62	510	1.58								
W_5 D-602	8,000	0.05	0	430	2.42			475	3.0	520	4.1			580	8.26	616	11.1	690	1.92
			10					473	4.64	520	4.56			580	4.32	616	4.88		
			30					471	4.42	508	3.94			570	2.64	620	2.44		
W_6 D-603	18,000	0.05	0	430	3.76					514	5.60					615	9.36	690	3.08
			10			460	5.16	476	5.34	514	5.88			580	4.76	615	5.40		
			30					470	5.26	506	5.20			572	2.94	620	2.68		
			0	430				484								614			
Cholesterol acetate pure	....	...	10	428	....	...	...	484	...	...	...	...	...	...	...	614	...	...	...
			30	428				484								614			

bands for a very pure sample of cholesterol acetate free from 7-dehydrocholesterol. The band at 690 mμ is now attributed (*2*, *4*, *9*) to vitamin A_2, the maxima at 620 and 580 mμ to vitamin A_1, the one at 500 to 520 mμ to vitamin D, and those at 480 and 430 mμ, respectively, to cholesterol. The maxima at 545 and 455 mμ, respectively, are not yet well understood. Cholesterol shows an additional maximum (see Figure 4) at 614 mμ which may interfere with the 620 mμ vitamin A_1 maximum. However, this maximum is rather weak and does not appear prominently during the early stages of the measurements.

In Table IV the potencies are calculated by three different methods from the extinction coefficients, using the CR-503 sample as the standard of reference. In the first method no correction was made. In the second method, however, a correction was made for the presence of sterols by subtracting from $E^{1\%}_{1\text{ cm.}}$ at 500–520 mμ, using the 10-minute curve, one half the value of $E^{1\%}_{1\text{ cm.}}$ at 480 mμ, using the 30-minute curve. This choice is based on the fact that the maximum of the cholesterol band develops much more slowly than that of the vitamin D band, and in some preliminary studies this maximum was found to be about twice that at 500 mμ.

In the third method a correction for the presence of vitamin A was introduced. This correction is not a simple factor but increases more rapidly than the concentration of vitamin A. A graphic method was finally used to obtain the correction which is plotted against the $E^{1\%}_{1\text{ cm.}}$ values of the vitamin A maximum at 620 mμ, us-

TABLE IV. COMPARISON OF BIOLOGICAL WITH SPECTROSCOPIC POTENCIES

Oil Sample	Biological Potency	Method I		Method II		Method III	
		$E^{1\%}_{1\text{ cm.}}$	Potency	$E^{1\%}_{1\text{ cm.}}$	Potency	$E^{1\%}_{1\text{ cm.}}$	Potency
	U. S. P. units/g.		*U. S. P. units/g.*		*U. S. P. units/g.*		*U. S. P. units/g.*
ACF-CR 503	50,000	17.77	50,000	10.42	50,000	9.39	50,000
ACF-CR 726	18,000	6.16	17,450	3.21	15,300	3.03	16,100
ACF-CR 1614	50–100	0.7	1,975	0.27	1,290	0.24	1,270
ACF-CR 1615	50–100	1.52	4,300	0.54	2,590	0.49	2,600
ACF-CR 1616	10,000	32.7	92,300	18.9	90,000	1.5	8,000
ACF-CR 1617	30,000	18.3	51,700	11.13	53,400	10.0	53,200
ACF-CR 1618	42,500	22.5	63,500	14.9	71,500	12.0	63,800
ACF-CR 1619	50,000	16.4	46,300	10.6	50,900	9.5	50,500
ACF-CR 1620	75,000	28.4	80,200	17.2	82,500	15.2	70,300
W_1-598	275	0.49	1,379	0.17	815	0.15	800
W_2-599	800	1.36	3,826	0.67	3,200	0.62	3,300
W_3-600	1,500	1.31	3,680	0.33	1,580	0.29	1,540
W_4-601	3,500	1.76	4,950	0.95	4,560	0.86	4,580
W_5-602	8,000	4.56	12,800	2.35	11,270	2.0	10,650
W_6-603	18,000	5.88	16,580	3.25	15,500	3.15	16,800

ing the zero-minute curve. The resulting curve is shown in Figure 5. The correction so obtained is subtracted from the $E^{1\%}_{1\text{ cm.}}$ values obtained by the second method. The corrected $E^{1\%}_{1\text{ cm.}}$ values together with the potencies obtained from these values are shown in the last two columns of Table IV.

A number of measurements were made on the nonsaponifiable fractions of several fish liver oils, but the results were less satisfactory than those given in Table IV.

Maleic Anhydride Procedure

Although the foregoing method seems to give somewhat satisfactory results, at least with high-potency oils, it is not universally applicable, as it is necessary to have spectroscopic equipment to measure the visible region of the spectrum accurately and rapidly. It also depends upon a number of corrections which make the method cumbersome. In view of these objections the authors developed the maleic anhydride procedure in which most of the substances that interfere with the vitamin D band are removed.

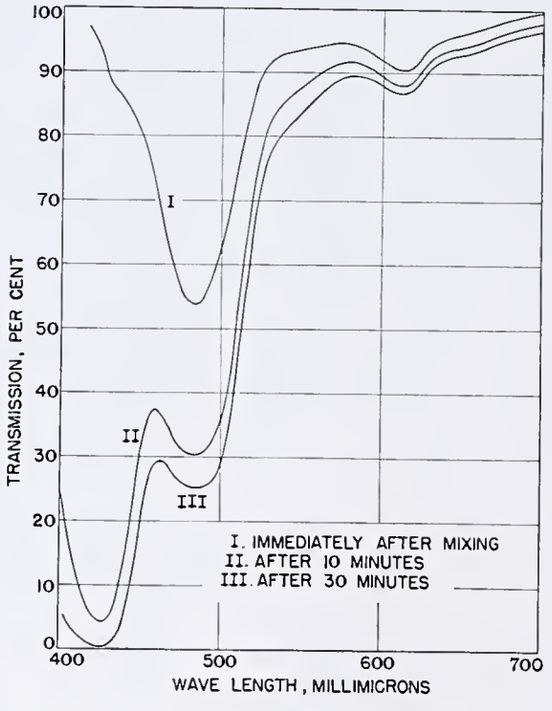

FIGURE 4. TRANSMISSION SPECTRUM OF ANTIMONY TRICHLORIDE COLOR OF PURE CHOLESTEROL ACETATE

A quantity of the fish liver oil in the neighborhood of 1 gram is accurately weighed and saponified in the usual manner. The nonsaponifiable matter is dried by distilling from it, under reduced pressure, an absolute alcohol–benzene mixture. To remove the greater portion of the sterols, the dried residue is then dissolved in about 5 cc. of pure methyl alcohol and the mixture is cooled to −10° to −15° C., then filtered at this temperature. The alcohol is completely removed from the filtrate, the residue is dissolved in about 5 cc. of freshly purified 1,4-dioxane (free from peroxides), and to it is added about 0.2 gram of maleic anhydride in about 2 cc. of dioxane. The maleic anhydride is more effective if it is freshly prepared by distilling in a moderate vacuum a 1 to 1.5 mixture of maleic acid and phosphorus pentoxide. The dioxane mixture is heated on the water bath for 1 hour, then cooled, 10 cc. of 0.5 *N* alcoholic potash are added, and it is allowed to stand at room temperature for 5 to 10 minutes. Fifteen cubic centimeters of water are then added and the mixture is extracted several times with pure ether; the ether extracts are washed twice with water, dried over anhydrous sodium sulfate, and filtered, and the ether is removed under reduced pressure.

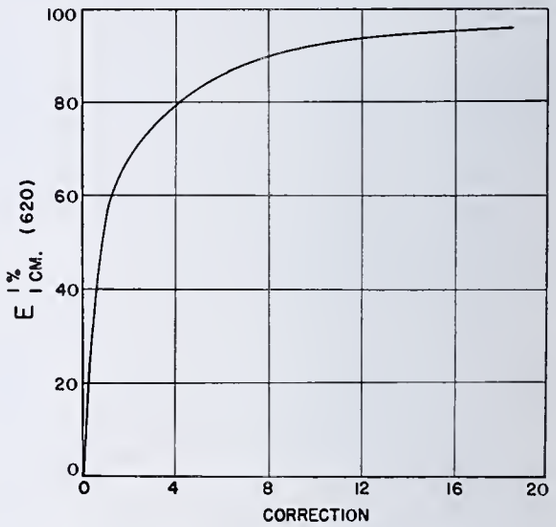

FIGURE 5. CORRECTION FOR PRESENCE OF VITAMIN A

Plotted against $E^{1\%}_{1\text{cm.}}$ at 620 mμ for 0-minute curve

Finally, the vitamin D in the residue is estimated spectrophotometrically, using the method described above.

The destruction of vitamin A by the maleic anhydride procedure is best illustrated by Figure 6. Three equal samples of fairly pure vitamin A were taken, two of which were heated on the water bath with maleic anhydride in dioxane in an atmosphere of nitrogen for 15 and 60 minutes, respectively. Transmission spectra were then taken of both the treated and untreated samples. The vitamin A absorption maximum was reached 3 and 10 minutes after mixing (lower and upper curves). It may be seen that 1-hour treatment with maleic anhydride destroys vitamin A almost completely, and the corresponding 10-minute curve, the standard time adopted for vitamin D measurements, shows almost zero vitamin A concentration. Figure 7 shows the 3- (I) and 10-minute (II) curves of one of the fish liver oils having moderate concentration of vitamin A after the treatment with maleic anhydride. Both curves show the complete absence of vitamin A.

Carotenoids having conjugated systems of double bonds and possibly 7-dehydrocholesterol are also removed by the maleic anhydride treatment. Although vitamin D contains a conjugated system of double bonds, it is known to react more sluggishly with maleic anhydride than its precursors (*17*).

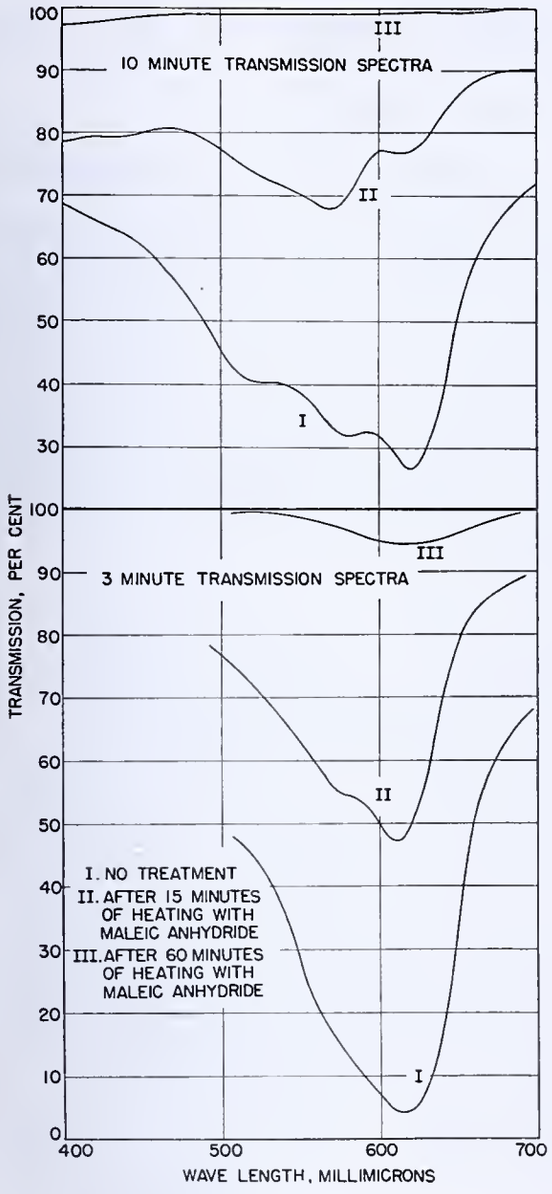
10 MINUTE TRANSMISSION SPECTRA
3 MINUTE TRANSMISSION SPECTRA
TRANSMISSION, PER CENT
WAVE LENGTH, MILLIMICRONS
I. NO TREATMENT
II. AFTER 15 MINUTES OF HEATING WITH MALEIC ANHYDRIDE
III. AFTER 60 MINUTES OF HEATING WITH MALEIC ANHYDRIDE

FIGURE 6. EFFECT OF MALEIC ANHYDRIDE ON PURE VITAMIN A

Table V shows the potencies and the $E_{1\text{ cm.}}^{1\%}$ values at 500 mμ of six fish liver oils obtained by the maleic anhydride procedure. The potencies referred to calciferol are slightly higher than the biological potencies, while those referred to sample 1619 are in close agreement with the biological.

TABLE V. POTENCIES OBTAINED BY MALEIC ANHYDRIDE PROCEDURE

Oil Sample	Biological Potency	$E_{1\text{ cm.}}^{1\%}$	Potency Referred to Calciferol	Potency Referred to 1619
	U. S. P. units/g.		*U. S. P. units/g.*	*U. S. P. units/g.*
1616	10,000	0.235	10,700	9,550
1617	30,000	0.778	35,000	31,000
1618	42,500	1.067	48,000	44,000
1619	50,000	1.23	55,900	50,000
1620	75,000	1.775	80,000	72,100
1793	45,000	1.135	51,000	46,100

Summary

Two independent physicochemical methods have been developed for the quantitative estimation of vitamin D in fish liver oils. One is based on the spectrophotometric estimation of $E_{1\text{ cm.}}^{1\%}$ values at 500 to 520 mμ of the antimony trichloride color of various fish liver oils. Corrections have been made for the presence of sterols and vitamin A, both of which seem to interfere with the vitamin D absorption band.

The second method is based on a chemical treatment of the nonsaponifiable fraction of fish liver oils with maleic anhydride to destroy vitamin A, carotenoids, and possibly 7-dehydrocholesterol. The vitamin D in the treated nonsaponifiable portions is then estimated spectrophotometrically as in the first method. The results obtained seem to be in fair agreement with the biological.

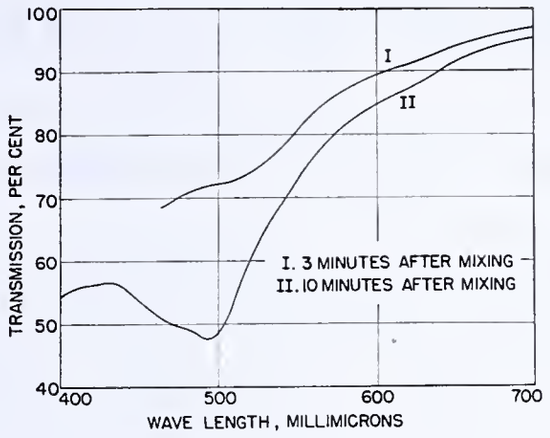

FIGURE 7. EFFECT OF MALEIC ANHYDRIDE ON VITAMIN A CONCENTRATION OF NONSAPONIFIABLE FRACTION OF A FISH LIVER OIL

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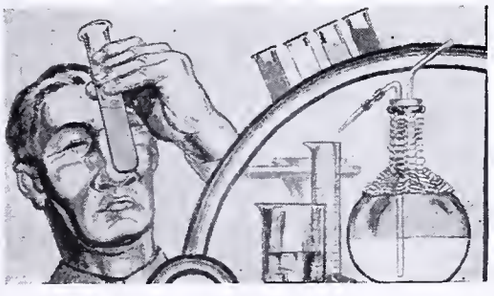
END OF SYMPOSIUM



Optical Activity of Quinine and Some of Its Salts

In Mixtures of Water and Ethyl Alcohol

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IN SPITE of the many years during which quinine has been a commonly used therapeutic agent, exact determinations of its physicochemical properties are frequently lacking in the literature. During the development of an improved microprocedure for the determination of quinine in blood and urine the authors found need for an independent method of determination which could be used on a macro or semimicro scale and used for this purpose the optical activity of quinine or its salts. The use of such a procedure on a semimicro scale has necessitated a search for those conditions under which the highest optical activity could be attained.

The bibliographies provided by Allen (*1*), Schmidt and Grafe (*8*), and other books on alkaloids include many references to the optical activity of quinine and its salts but with the partial exception of the work of Oudemans (*7*), systematic studies of the effect of progressive neutralization of the free base, the effect of progressive change of solvent (ethyl alcohol, water), etc., under exact temperature control and with highly purified samples are largely lacking. Some data of this sort were reported by Hesse (*3*, *4*) and, more recently, by Schoorl (*9*), Dietzel and Soellner (*2*) and Lapp (*5*). The data in the present paper are not in any essential way in contradiction with those of the above authors but constitute an amplification and a partial confirmation of their work. Oudemans' determinations, while the most complete in the literature, frequently fail to cover the range desired for present purposes.

All determinations of optical activity were carried out at 25° ± 0.5° C. using a Schmidt and Haensch half-shadow polarimeter reading to ±0.01°, an electric sodium lamp providing practically monochromatic D light, and 4-dm. tubes.

Quinine as the free base was prepared by decomposition of either hydrochloride or sulfate with excess ammonia and crystallization at 0° C. from the water solution. Samples of the free base prepared from both salts gave identical optical activities.

Quinine dihydrochloride was prepared by recrystallization from absolute alcohol. The resulting addition compound with alcohol was then decomposed by drying at room temperature *in vacuo* and then briefly at 110° C. This product gave rotation values practically identical with those of the U. S. P. product dried *in vacuo* over phosphorus pentoxide at room temperature.

Quinine sulfate was prepared by several successive recrystallizations of the U. S. P. product from hot water and the product was dried to constant weight *in vacuo* over phosphorus pentoxide. Constancy of optical activity was used as the criterion of complete purification with both the free base and its salts. In all the following data, specific rotations are uniformly expressed in terms of the free base.

TABLE I. SPECIFIC ROTATION OF QUININE FREE BASE, QUININE DIHYDROCHLORIDE, AND QUININE SULFATE

(In varying percentages of water and alcohol)

Alcohol (by Volume) %	Quinine Free Base $-[\alpha]_D^{25}$	Quinine Sulfate $-[\alpha]_D^{25}$	Quinine Dihydrochloride $-[\alpha]_D^{25}$
0	...	176	270
6.16	...	...	272
8.60	...	187	...
10.0	120	...	...
18.5	...	202	...
20.0	138	...	277
30.0	155	218	280
40.0	171	225	277
50.0	179	233	273
60.0	181	237	267
70.0	180	233	252
80.0	178	221	240
90.0	172	214	222
100.0	168	206	207

Rotation of Quinine Free Base, Hydrochloride, and Sulfate in Water-Alcohol Mixtures

A stock solution containing 2.5000 grams of the recrystallized free base made up to 100 ml. in freshly prepared absolute ethyl alcohol was used for the water-alcohol curve of the free base. Five-milliliter aliquots were measured into 50-ml. volumetric flasks, measured amounts of water were added from a buret, and the flasks were made up to volume with absolute alcohol. All concentrations of quinine were therefore 0.250 gram of free base per 100 ml. with the exception of the two solutions containing only 10 and 20 per cent of alcohol (by volume), respectively. These, because of the limited solubility of the free base, contained higher dilutions of the latter.

Similar procedures were used with both the sulfate and the hydrochloride. In all cases the stock solution was made up in absolute ethyl alcohol.

Table I shows the results obtained with quinine free base, the dihydrochloride, and the sulfate. All figures for $[\alpha]_D^{25}$ refer to the free base regardless of the salt used.

It is obvious that the same amount of quinine base produces a much higher rotation as the hydrochloride than as the sulfate or as the free base. Furthermore, for quinine hydrochloride the curve of $[\alpha]_D^{25}$ with alcohol concentration reaches a flat maximum at about 30 per cent alcohol by volume and gives, at the maximum, a specific rotation of −280. Inaccuracies in the dilution of the alcohol-water mixture used as solvent would therefore have little effect on the final reading. With both quinine sulfate and the free base the maximum of the curve is reached at about 60 per cent alcohol with lower specific rotation at these points.

TABLE II. EFFECT ON OPTICAL ACTIVITY OF PROGRESSIVE NEUTRALIZATION OF QUININE BASE WITH HYDROCHLORIC AND SULFURIC ACIDS

Hydrochloric Acid Molar ratio of HCl to quinine	$-[\alpha]_D^{25}$	Sulfuric Acid Molar ratio of H_2SO_4 to quinine	$-[\alpha]_D^{25}$
0	153	0	177
0.258	175	0.028	184
0.516	198	0.141	200
0.774	216	0.283	217
1.032	236	0.424	234
1.290	252	0.565	246
1.548	270	0.706	252
1.806	278	0.848	256
2.064	283	0.989	257
2.322	286	1.130	258
2.580	289		
2.838	290		

Neutralization Curves of Quinine Base with Sulfuric and Hydrochloric Acids

To determine the effect of progressive neutralization of the free base, fixed amounts were neutralized by addition of varying amounts of standard acid (hydrochloric or sulfuric). These solutions were so prepared as to contain that proportion of water and ethyl alcohol which was shown in Table I to give the maximum specific rotation of their respective salts (30 per cent alcohol for the hydrochloride and 60 per cent alcohol for the sulfate). The extent of neutralization is indicated by the molar ratio of acid to quinine. This ratio is obviously 0.5 for the sulfate and 2.0 for the hydrochloride in the case of the purified salts used in the measurements of Table I.

Table II shows the data obtained expressed as the specific rotation (as free base) for various molar ratios of acid to qui-

nine. All solutions contained 0.250 gram of quinine per 100 ml. of solution. All points of the hydrochloric acid curve were determined in 30 per cent alcohol and all of the sulfuric acid curve in 60 per cent alcohol.

The data in Table II show for both acids only a smooth curve with no breaks at any stoichiometric ratios. They show, however, why somewhat higher rotations have been obtained when excess acid has been added to either the sulfate or the dihydrochloride. The report of Liquier (*6*) concerning plateaus in the curve of optical activity *vs.* pH corresponding to the neutral and basic quinine sulfates has not been confirmed by the authors.

Summary

Data are presented to show the variation in optical activity of quinine as free base, dihydrochloride, and sulfate in mixtures of water and ethyl alcohol, and the variation as the free base is progressively neutralized with hydrochloric and sulfuric acids, each in that water-alcohol solution which gives the maximum rotation for each salt.

Acknowledgment

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Effect of Temperature of Alcohol in Determination of Potash in Fertilizers

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FOR a number of years the referee on potash in fertilizers of the Association of Official Agricultural Chemists has recommended investigation of the solubility of potassium chloroplatinate in alcohol and acid-alcohol. Pierrat gives the solubility of potassium chloroplatinate in alcohol at 14° C. (*3*) but makes no reference to the solubility at the higher temperatures at which most laboratory work is done. Allen reports on the greater solubility of potassium chloroplatinate in 80 per cent alcohol than in 95 per cent alcohol (*1*) but makes no reference to the temperature of the alcohol used. Archibald, Wilcox, and Buckley give the solubility of potassium chloroplatinate in alcohol-water mixtures of various alcohols at 20° C. (*2*) but do not mention the solubility in acid-alcohol. This work, while important in itself, has not been inclusive enough to encompass the conditions existing in the determination of potash by the official method, as, for example, the effect of temperature on the solubility of potassium chloroplatinate in alcohol and acid-alcohol.

During the summer of 1939 a large number of low-analysis potash fertilizers analyzed in the authors' laboratory were found to be running below guarantee. The daily temperatures during this period were unusually high but after cooling the alcohol to about 18° C. the results were from 0.1 to 0.3 per cent higher. Since a rise of about 8° C. was noted upon addition of concentrated hydrochloric acid to alcohol (when added at the rate of 0.6 ml. of hydrochloric acid to 6 ml. of alcohol) and the temperature remained above room temperature for the 15-minute extraction period, it was found advisable to mix the acid and alcohol and cool before adding it to the potassium chloroplatinate. Subsequently the effect of temperature on the solubility of potassium chloroplatinate in acid alcohol was studied. Known concentrations of pure potassium chloride were used for samples in place of commercial fertilizers in order to avoid other sources of error in the potash determination.

Table I. Loss of Potassium Chloroplatinate by Extraction with Acid-Alcohol

(0.1-gram portions of K_2PtCl_6 used)

Treatment	K_2O Lost 18° C.	38° C.	48° C.
	Mg.	*Mg.*	*Mg.*
Mixed acid-alcohol	5.1	8.2	10.8
Acid and alcohol separate	7.6	9.7	11.6

Procedure

The study is divided into two steps.

1. Extraction of weighed amounts of pure potassium chloroplatinate with definite volumes of acid and alcohol under controlled temperature conditions. Tenth-gram portions of pure potassium chloroplatinate were transferred to 250-ml. beakers. To these were added 137.5 ml. of acid alcohol at a definite temperature for the mixed acid-alcohol determinations and 125 ml. of alcohol and 12.5 ml. of concentrated hydrochloric acid for the determinations in which the acid and alcohol were added separately. The mixtures were stirred for 15 minutes at controlled temperatures. Finally the determinations were filtered into sintered-glass crucibles and washed with 137.5 ml. more of alcohol at corresponding temperatures. Table I gives the amounts of potassium chloroplatinate lost by extraction.

2. Determination of the effect of the temperature of acid-alcohol on the solubility of potassium chloroplatinate precipitated from two concentrations of pure potassium chloride. Two concentrations of potassium chloride were used. The first contained 6.25 grams of potassium chloride per liter, made to volume at 4° C. and kept at that temperature till used. The theoretical value of this solution is 0.3948 per cent K_2O. The second solution contained 12.5 grams of potassium chloride per liter and the theoretical K_2O value for this solution is 0.7896 per cent.

Aliquots of the above concentrations (25 ml.) were measured into platinum dishes, measured amounts of chloroplatinic acid were added, and the solutions were evaporated to a thick paste on the steam bath. The dishes were then removed and to each dish were added 6 ml. of 83 per cent alcohol and 0.6 ml. of concentrated hydrochloric acid for the determinations in which the acid and alcohol were added separately or a mixture of 6 ml. of alcohol and 0.6 ml. of acid for the mixed acid-alcohol determinations. The samples were extracted for 15 minutes at 18° or at 38° C. The temperatures in each case were controlled by a constant-temperature bath. At the end of the 15-minute extraction, the samples were washed with 125 ml. more of 83 per cent alcohol adjusted to the corresponding temperature. It was estimated that 125 ml. of alcohol were normally used in the potash deter-

mination previous to the ammonium chloride washing, which was omitted in this case as no magnesium salts were present in the determinations.

All filtrations were made in the same set of sintered-glass crucibles (Jena B. G. 3). Actual potash results were determined by difference (the potassium chloroplatinate being dissolved out and the crucibles weighed back). Table II gives the solubility loss of potassium chloroplatinate at different temperatures.

TABLE II. SOLUBILITY LOSS OF POTASSIUM CHLOROPLATINATE

(Calculated as per cent K_2O in acid-alcohol at 18° and 38° C.)

Treatment	No. of Analyses Averaged	K_2O Theoretical Value	At 18° C. K_2O found	At 18° C. K_2O lost	At 38° C. K_2O found	At 38° C. K_2O lost
		%	%	%	%	%
Mixed acid-alcohol	50	0.7896	0.7851	0.57	0.7760	1.72
Acid and alcohol separate	50	0.7896	0.7794	1.29	0.7640	3.24
Mixed acid-alcohol	50	0.3948	0.3907	1.04	0.3848	2.53
Acid and alcohol separate	50	0.3948	0.3852	2.43	0.3783	4.18

Discussion of Results

Tables I and II indicate that a rise in temperature of the alcohol is accompanied by an increase in solubility of the potassium chloroplatinate. Although the differences in the amount of potassium chloroplatinate extracted when the acid and alcohol were added separately and when the mixed acid-alcohol was added are small, there is a significantly greater solubility with the former. This may be due to the increased temperature resulting from the mixing of the acid and alcohol.

When alcohol and acid are mixed in the proportions outlined in the official method for the determination of potash in fertilizers, there is a sharp rise in temperature of about 8° C. which does not entirely disappear at the end of the 15-minute extraction period. This indicates that it is advisable to mix the acid and alcohol and to control the temperature during the 15-minute extraction.

The percentage losses in Table II are greater at the lower concentration of K_2O. This may be due to the solvent action of the alcohol on the smaller crystals of potassium chloroplatinate which are formed in the lower concentration of K_2O. The small crystals present greater surface area than the larger crystals formed with the higher concentrations and therefore have a greater solubility.

Conclusions

There is a definite increase in temperature when the acid is added to the alcohol, as outlined in the A. O. A. C. method for the determination of potash in fertilizers, which gives an error particularly under summer laboratory conditions. This can be prevented by mixing the acid and alcohol beforehand. In order to eliminate the effect of temperature it is advisable to cool the acid-alcohol mixture as well as the wash alcohol before using.

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The Reducing Properties of *l*-Sorbose

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WITH the current commercial use of *l*-sorbose it seemed desirable to determine its reducing property by one of the standard methods and to compare this property with that of the other available ketohexose, fructose.

The *l*-sorbose was prepared by the method of Fulmer and others (*1*) and yielded eight-sided crystals in the orthorhombic system. The sugar was not fermented by *Saccharomyces cerevisiae* (a commercial baking strain and one isolated from cheese) nor by two strains of Torula. The product lost no weight upon heating for 2 hours in a vacuum oven at 70° C. Various samples melted with darkening from 162.4° to 164.2°, with a mean value of 163.5° (corrected), and the osazone melted at 163° (corrected). A 6 per cent solution showed $[\alpha]_D^{20} = -42.9°$ when corrected for temperature and concentration by the formula of Smith and Tollens (*4*). A recent value by Pigman and Isbell (*3*) records $[\alpha]_D^{20} = -43.4°$ for a 12 per cent solution.

TABLE I. CERIC SULFATE EQUIVALENTS AT VARYING CONCENTRATIONS OF SORBOSE

Sorbose Concentrations x		0.02 N Ceric Sulfate, y	Factor, x/y
Mg./5 ml.	%	*Ml.*	
0.05	0.001	0.20	0.250
0.10	0.002	0.19	0.526
0.50	0.01	1.21	0.413
1.00	0.02	2.46	0.407
5.00	0.10	11.40	0.439
10.00	0.20	20.29	0.493
15.00	0.30	28.47	0.527
20.00	0.40	36.46	0.549
25.00	0.50	43.94	0.569
30.00	0.60	51.18	0.586
35.00	0.70	58.42	0.599

TABLE II. CERIC SULFATE EQUIVALENTS AT VARYING CONCENTRATIONS OF FRUCTOSE

Fructose Concentration x		0.02 N Ceric Sulfate, y	Factor, x/y	Fructose / Sorbose
Mg./5 ml.	%	*Ml.*		
1.00	0.02	2.77	0.361	1.13
5.00	0.10	12.24	0.408	1.07
10.00	0.20	21.66	0.462	1.08
15.00	0.30	30.88	0.486	1.08
20.00	0.40	40.01	0.500	1.10

The reducing power was determined by the method of Hildebrand and McClellan (*2*). In each case the desired quantity of the sugar was contained in 5 ml. of solution which was allowed to react with 10 ml. of the alkaline ferricyanide solution. The values are corrected for the blank which was determined daily and never exceeded 0.7 ml. of a 0.01773 N ceric sulfate solution. All data are recorded as equivalents of 0.02 N ceric sulfate and represent the mean of at least triplicate determinations.

In a similar way the equivalent reducing power of a sample of Pfanstiehl's C. P. special fructose was determined for comparative purposes. The data are recorded in Table II, where the last column indicates the relative reducing properties of the two ketosugars.

The data indicate that the direct titration with ceric sulfate of the formed ferrocyanide is satisfactory for sorbose in the concentration range of from 0.01 to 0.70 per cent. At concentrations below this range the accuracy is considerably reduced, and at higher concentrations the ferricyanide is completely reduced.

The results obtained in comparing the reducing properties of fructose and sorbose bear out the hypothesis of Sobotka and Reiner (*5*) that the configuration about the third and fourth carbon atoms of the hexose sugars is the important factor in determining the reducing properties, and that, if the configurations of the hydroxyl groups on carbon atoms 3 and 4 of two sugars are similar, these sugars will have approximately the same reducing properties. Fructose and sorbose have the same configuration on these carbon atoms and their reducing powers are of the same order of magnitude.

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Determining Copper and Nickel in Aluminum Alloys

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IN CONJUNCTION with the spectrographic analysis of aluminum alloys, this laboratory performs the routine chemical determination of copper in a large number and variety of aluminum alloys in which the copper concentration varies from 0.2 to about 10 per cent. The determination of nickel in aluminum alloys in amounts up to 2.5 per cent is also required.

The commonly used methods for copper (*2*, *4*, *5*) call for solution of the alloy in caustic solution and subsequent filtration. This leaves copper with other caustic-insoluble material on the filter, from which it is then removed with hot dilute nitric acid and determined by electrodeposition. This procedure serves only to separate the alloying elements (except zinc) from the bulk of the aluminum, and as commonly employed involves the danger of loss of material by spray in the sometimes violent reaction which occurs when the precipitate is washed from the filter with hot acid.

The present work was undertaken in an attempt to find suitable conditions for the direct electrodeposition of copper without preliminary separation of the bulk of the aluminum. Since aluminum alloys dissolve very slowly in acids other than hydrochloric and since hydrochloric acid is undesirable because it must be removed before electrolysis, the procedure used involved solution of the alloy in caustic solution, and, after dilution, addition of an excess of nitric acid. This method has found use in this laboratory in other cases where chlorides are objectionable—for instance, in the determination of manganese it is much to be preferred to the very slow method of dissolving in a mixture of nitric and sulfuric acids. This treatment gives complete solution of the alloy, except that with high-silicon alloys some silicon remains undissolved. It was found that this introduced no error in the determination of copper. If appreciable quantities of tin or other elements which interfere with the electrodeposition of copper are present, this method may not be used.

This method, since it cuts manipulation to a minimum, reduces the time required for a complete determination to less than one third of that required by the longer method. For a batch of twelve, the time elapsed between the completion of weighing the samples and the point at which they are ready to be electrolyzed is no more than 30 minutes, as compared with 1.5 to 2 hours required by the longer method. The sources of error due to incomplete solution of copper from the filter paper and the possibility of loss by spray are eliminated and the method has the further desirable feature of being carried out completely in a single vessel.

After deposition of the copper, the electrolyte can be used for the determination of nickel.

The method has been in continued use in this laboratory for some months and has proved satisfactory for routine analysis.

Table I. Determination of Copper and Nickel[a]

Alloy No.	Alloying Elements	Copper Found		Nickel Found	
		Short method	Long method	Short method	Referee method
	%	%	%	%	%
1	1.5 Zn, 1.5 Fe	7.68	7.67	..	..
2	0.5 Mn, 0.5 Mg	4.07	3.97	..	..
3	0.5 Mn, 1.5 Mg	4.68	4.55	..	..
4	0.8 Mn, 0.8 Si	4.58	4.54	..	..
5	0.2 Mg, 1.2 Fe	9.84	9.78	..	..
6	0.8 Si, 0.6 Fe	4.43	4.31	..	..
7	1.5 Si, 1.2 Fe	7.18	7.16	..	..
8	12.0 Si, 0.8 Fe, 1.0 Mg	0.73	0.76	2.38	2.43
9	1.5 Mg	3.86	3.86	1.75	1.71
10	0.9 Si	5.77	5.72	0.79	0.81
11	5.0 Si, 0.5 Mg	1.34	1.29	..	..
12	2.5 Si	4.45	4.42	..	..

[a] All values except referee nickel values are averages of two or more determinations.

Proccdures

Copper. A 1-gram sample is transferred to a 200-ml. electrolytic-type beaker and treated with 15 ml. of 20 per cent sodium hydroxide. If the reaction is too vigorous, water is added. After the reaction subsides, the beaker is heated on the steam bath until the reaction is completed, as indicated by the cessation of effervescence. The solution is then diluted to 100 ml. with hot water and stirred thoroughly, and 30 ml. of 1 to 1 nitric acid are added. This causes precipitation of gelatinous aluminum hydroxide, which redissolves on stirring and warming on the steam bath. Then 2 ml. of 1 to 1 sulfuric acid are added, the solution is stirred, and after cooling to room temperature is electrolyzed at 2 to 3 amperes, using a gentle air stream as a stirring device. A platinum gauze cathode 45 mm. in diameter and 50 mm. in height is used.

If the acid concentration is not sufficiently high, a white deposit of aluminum hydroxide will adhere to the copper deposit. This will be avoided if the quantities recommended above are used.

Nickel. The electrolyte from the copper determination is transferred to a 400-ml. beaker and 25 ml. of 25 per cent tartaric acid are added. The solution is then made ammoniacal and warmed on the steam bath. In order to facilitate the removal of silica, 5 ml. of a 5 per cent zinc chloride solution are added and the solution is stirred and warmed further. It is then filtered into a 600-ml. beaker and the residue is washed with hot water. The filtrate is made slightly acid with dilute hydrochloric acid and 20 ml. of 1 per cent alcoholic dimethylglyoxime solution are added (for samples containing over 2 per cent of nickel 5 ml. more are used for each additional 1 per cent). Dilute ammonia is then

added while stirring until a slight excess is present. The mixture is warmed on the steam bath for an hour, filtered onto a weighed Gooch or sintered-glass crucible, washed with hot water and once with methanol, and dried at 110°.

Results

To check the method twelve different types of aluminum alloys were analyzed for copper by the procedure herein described and by the longer method involving separation from aluminum (*2*). Three were nickel-bearing and were analyzed for nickel both by the procedure here described and by a referee method (*3*). The results are shown in Table I.

The first alloy listed (No. 1) is National Bureau of Standards light aluminum alloy standard sample No. 86, the copper value of which is reported as 7.66 per cent.

Discussion

The values for copper obtained by the shorter method are in most cases slightly higher than those obtained by the long method. Hence, to check on the accuracy of the shorter method, in ten determinations the deposited copper was dissolved from the electrodes and redeposited. In all cases the weights of copper changed by no more than 0.5 mg. and the average change was 0.3 mg. As this change is of the same order of magnitude as the experimental error, it is believed that the difference in the values obtained by the two methods is due to the slight solubility of copper in the alkali used in making the aluminum separation in the longer method (*1*).

To demonstrate this, the filtrates from the treatment of 1-gram samples of alloys 5 and 11 (Table I) by the longer procedure (*2*) were concentrated to 35 ml. and were then analyzed spectrographically by comparison with standards. This examination showed that the filtrate from alloy 5 contained between 0.5 and 1.0 mg. of copper, and the filtrate from alloy 11 contained about 1.0 mg. of copper. A blank treated in the same way showed no appreciable amount of copper. Since alloy 5 contains more than seven times as much copper as alloy 11, it is indicated that the amount of copper passing into the alkali filtrate is not a function of the copper content of the alloy but may depend on other factors, such as the amounts of other alloying elements present, the physical subdivision of the sample, and the length of time of treatment prior to filtration.

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This communication is not to be construed as an official publication of the U. S. Navy Department.

Simplification of the Petering-Wolman-Hibbard Method for Determination of Chlorophyll and Carotene

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IN VIEW of the interest shown in the method for the determination of chlorophyll and carotene in green plant tissue recently published by Petering, Wolman, and Hibbard (*3*) it seems advisable to make generally available a subsequent simplification of the barium hydroxide technique for removing chlorophyll from the acetone extracts of plant tissue.

Instead of adding the solid barium hydroxide octahydrate reagent to the aqueous-acetone extract, a saturated solution of barium hydroxide is added to the pure acetone extracts in suitable amount to remove all of the chlorophyll, and this mixture is then treated as directed in the original procedure. This technique eliminates the necessity of preparing an active solid reagent from anhydrous barium hydroxide, of having to handle finely divided barium hydroxide octahydrate, or of keeping the solid reagent free from carbonate. During the preparation of the solution the carbonate is removed because of its insolubility, and the addition of dissolved barium hydroxide to the acetone extract produces a very finely divided precipitate of barium hydroxide octahydrate which is extremely active.

This technique has been extensively used by Petering, Morgal, and Miller (*2*) in their work on the isolation of carotene from green plant tissue and its possible use in the analytical procedure has been suggested by Benne, Wolman, Hibbard, and Miller (*1*). Peterson (*4*) recently has pointed out that this general technique is satisfactory. However, no specific directions have been given which could be easily followed in an analytical method.

The following procedure is therefore suggested: Five grams of fresh plant tissue (or 1 gram of dried tissue) are ground and extracted with pure acetone and the extract is brought to a volume of 200 ml. A 100-ml. aliquot is taken and to it are added 15 ml. of saturated solution of barium hydroxide. This mixture is then refluxed for 30 minutes as in the original procedure, which results in the complete removal of chlorophyll from the extract.

Fifteen milliliters of a solution of barium hydroxide saturated at room temperature contain about 0.60 gram of barium hydroxide, which according to the data of Petering, Morgal, and Miller is sufficient to remove about 100 mg. of chlorophyll from an 85 per cent aqueous-acetone extract. Since 5 grams of fresh, highly pigmented plant tissue contain about 20 mg. of chlorophyll, it is obvious that the amount of barium hydroxide in the volume of solution recommended is adequate for its complete removal from the extract. This procedure, furthermore, automatically takes care of the water-acetone ratio which has been found very important to the efficiency of the barium hydroxide technique (*2*).

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Removal of Thiocyanate in the Detection of the Halides

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MANY methods have been proposed for the removal of thiocyanate, which is the most serious interference in the detection of the halides.

Sneed and Duschak (*8*) destroy the thiocyanate by igniting the silver halide precipitate and then fusing it with a mixture of sodium carbonate and potassium carbonate. Curtman and Schneiderman (*1*) also destroy the thiocyanate by igniting the silver precipitate. Both Curtman and Wikoff (*2*) and Dobbins and Ljung (*3*) remove thiocyanate by precipitating it as the cuprous salt. Noyes (*7*) oxidizes it with potassium permanganate, while MacAlpine and Soule (*6*) oxidize it by means of persulfate. Heisig and Heisig (*5*) present a modified procedure of Prescott and Johnson. Swift (*9*) oxidizes the thiocyanate by means of nitrate ion in a solution wherein the acidity is adjusted (using a sulfate-hydrosulfate buffer). In a previous paper (*4*) the authors proposed the use of concentrated sulfuric acid to destroy the thiocyanate.

Procedures for the detection of the halides involving the above methods for removing thiocyanates were found to be very time-consuming. Therefore, the desire for a rapid method of detecting thiocyanate and the halides when as little as 1 per cent of the total concentration is present, stimulated the authors to develop a simple, rapid, and efficient procedure for the removal of thiocyanate. In this new procedure a large part of the thiocyanate is removed with lead nitrate and the remainder destroyed by boiling with sodium nitrite in dilute nitric acid solution.

After the silver halide precipitate has been brought into solution by boiling with ammonia sulfide, the solution to be tested for halides is acidified and then rendered just alkaline. Lead nitrate is added to remove a large portion of the iodide and thiocyanate, since large amounts of iodide interfere with the test for thiocyanate (*4*). This provides a fixed concentration of anions in the form of a saturated solution of their lead salts except when the anions are present in small concentrations.

The precipitate is removed by centrifuging and a portion of the centrifugate is taken for the test for thiocyanate and iodide. Ferric nitrate and carbon tetrachloride in a dilute nitric acid solution are used to detect these anions. The remainder of the centrifugate is acidified with nitric acid, sodium nitrite is added, and the solution is boiled. In this manner iodide and thiocyanate are removed simultaneously. Potassium permanganate and carbon tetrachloride are then used to detect the bromide in a portion of this solution.

Using the procedure described below, only 1 ml. of 0.02 *M* permanganate was required to detect the bromide in all cases except where a large amount of bromide was present. In this instance the addition of potassium permanganate was discontinued after 3 ml. had been added, since sufficient bromide had already been oxidized.

The remainder of the solution (after boiling with nitrite) is used for detecting the chloride with silver nitrate. If bromide is present it is removed by boiling with concentrated nitric acid.

Procedure

Dilute the solution to be tested (the silver sulfide has been filtered off and the sulfide and ammonia removed by boiling) to 22 ml. in a 100-ml. beaker and add 3 *M* nitric acid dropwise and with constant stirring until the solution is just acid. Add 1.5 *M* sodium carbonate dropwise and with constant stirring until the solution is distinctly blue to nitrazine paper. Then add 12 ml. of *M* lead nitrate dropwise and with constant stirring. Stir for 2 minutes, transfer to a large centrifuge tube, and centrifuge.

A. DETECTION OF THIOCYANATE. To one quarter of the centrifugate add 3 ml. of 3 *M* nitric acid and 5 ml. of 2 *M* ferric nitrate. A blood-red solution shows the presence of thiocyanate.

B. DETECTION OF IODIDE. Transfer the solution of a 50-ml. glass-stoppered Erlenmeyer flask which contains 2 ml. of carbon tetrachloride. Let it stand for 3 minutes, shaking at 1-minute intervals. A violet-colored carbon tetrachloride layer shows the presence of iodide.

C. DETECTION OF BROMIDE. (1) *Thiocyanate and Iodide Present.* Under a hood transfer the remaining three quarters of the centrifugate to a beaker. Add 5 ml. of 3 *M* nitric acid and then 1.5 ml. of 6 *M* sodium nitrite dropwise and with constant stirring. Heat to boiling with constant stirring, boil for 2 minutes, cool, and filter off any precipitate.

(2) *Thiocyanate and Iodide Absent.* Add 5 ml. of 3 *M* nitric acid to the remaining three quarters of the centrifugate. To two thirds of the solution resulting from either (1) or (2) add 1 ml. of 15 *M* nitric acid and then 0.02 *M* potassium permanganate in 1-ml. portions until the solution remains pink or until a maximum of 3 ml. of permanganate has been added. Transfer to a 50-ml. glass-stoppered Erlenmeyer flask containing 1 ml. of carbon tetrachloride and shake. A yellow or brown carbon tetrachloride layer shows the presence of bromide.

D. DETECTION OF CHLORIDE. (1) *Bromide Present.* Dilute the remaining one third solution from C to 20 ml. and add with stirring 10 ml. of 15 *M* nitric acid. Heat to boiling with stirring and boil gently for at least 4 minutes and until all the bromine has been given off. Filter off any precipitate.

(2) *Bromide Absent.* To the remaining one-third solution from C add one tenth of its volume of 15 *M* nitric acid.

To the solution from (1) or (2) add 3 ml. of 0.5 *M* silver nitrate. Let stand for at least 5 minutes. A white precipitate shows the presence of chloride. Compare with a 1-mg. chloride control which has undergone the same procedure.

TABLE I. TEST ANALYSES

				Results			
CNS^-	I^-	Br^-	Cl^-	CNS^-	I^-	Br^-	Cl^-
Mg. per 3 ml.							
1	1	1	1	+	+	+	+
100	1	1	1	+	+	+	+
1	100	1	1	+	+	+	+
1	1	100	1	+	+	+	+
1	1	1	100	+	+	+	+
100	0	0	0	+	−	−	−
0	100	0	0	−	+	−	−
0	0	100	0	−	−	+	−
0	0	0	100	−	−	−	+

Test Analyses

The test analyses of Table I were carried out on 12 ml. of solution, using the above procedure.

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Determination of Formaldehyde with 5,5-Dimethylcyclohexanedione-1,3

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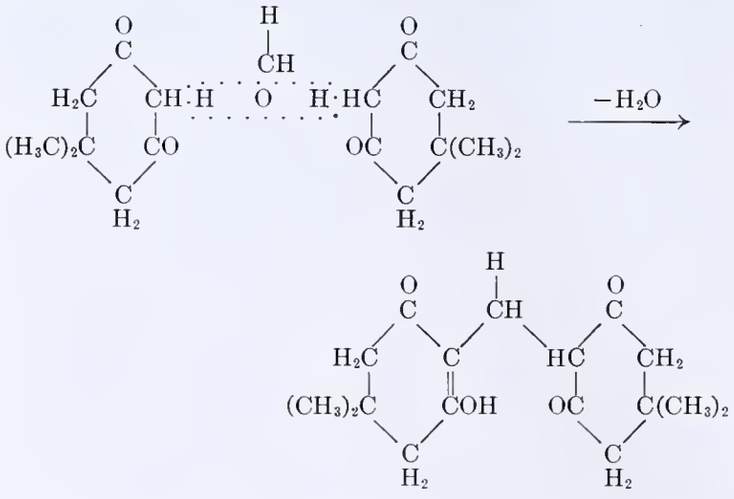
VORLÄNDER (*5*) and Ionescu and Bodea (*2*, *6*) found that for quantitative results the precipitation of formaldehyde with 5,5-dimethylcyclohexanedione-1,3, commonly called "dimedon", must be carried out in a neutral or very weakly acid solution. They did not, however, determine the influence of the hydrogen-ion concentration on the precipitation. The object of this investigation, therefore, was to find the effect of changing pH, to determine the optimum pH for the precipitation, and to study the determination of formaldehyde with dimethylcyclohexanedione in buffered solutions. This work was extended to finding the optimum pH for the precipitation of acetaldehyde and of propionaldehyde, and a comparison was made between the properties of these precipitates and that from formaldehyde.

Standard Formaldehyde Solution

A solution containing approximately 1 gram per liter of formaldehyde was prepared by adding 2.7 ml. of a 37 per cent formaldehyde solution (reagent quality) to distilled water and diluting to 1 liter. This solution was standardized by the method of Orchard (*4*), a 10-ml. sample being taken for the standardization. Assuming that 10 ml. of the solution contained 10 mg. of formaldehyde, the sample would require 0.107 gram of dimedon for a 15 per cent excess—two molecules of dimedon condense with one molecule of formaldehyde through loss of a molecule of water. Two 10-ml. samples of the formaldehyde solution were



withdrawn and each was added to 0.107 gram of dimedon dissolved in 50 ml. of water. The mixtures were shaken, allowed to stand overnight in stoppered flasks, and filtered through previously weighed sintered-glass crucibles. The precipitates and crucibles were dried in vacuum over phosphorus pentoxide to constant weight, and multiplication of weight of precipitate by 0.1027 (ratio of molecular weights) gave the amount of formaldehyde in the 10-ml. samples. The pH of the filtrate from a dimedon-formaldehyde precipitation made exactly as above was found to be 4.3.

Table I shows a comparison of the results with Orchard's method and with dimedon. From the mean the strength of the formaldehyde solution was taken to be 1.086 grams of formaldehyde per liter.

Determination of Optimum pH

A saturated solution of dimedon was prepared and filtered; 1 ml. of this solution contains approximately 0.004 gram of dimedon at room temperature. A formaldehyde solution of 0.1 gram per liter was prepared by diluting the standard in a volumetric flask. As buffers, Walpole's sodium acetate–hydrochloric acid mixtures (*1*) for pH 1.0 to 5.2, and Snell's monobasic phosphate–sodium hydroxide mixtures (*3*) for pH 5.8 to 7.0 were used. The method in general was to add 6.42 ml. (10 per cent excess) of the saturated dimedon solution to 250 ml. of the buffer in a 300-ml. Erlenmeyer flask and to this solution, by means of a precision pipet, add 25 ml. of the 0.1 gram per liter formaldehyde solution. The flask was stoppered and allowed to stand approximately 24 hours with occasional shaking. The solution was then filtered through a previously weighed sintered-glass crucible, and the precipitate and crucible were dried to constant weight at 55° to 60° C. In the process of filtering, a sample of the filtrate was caught in a test tube placed in the filter flask, and was set aside for several days. Further precipitation, if any, was noted, and then its pH was determined by means of a Youden hydrogen-ion apparatus using quinhydrone electrodes. As in the low pH range (2.9 to 4.2) precipitation was incomplete after 24 hours, several days were allowed for these precipitations.

The results of this experiment are plotted in Figure 1. Theoretically, 25 ml. of the 0.1086 gram per liter formaldehyde solution should have yielded 0.0264 gram of precipitate. The greatest amount of precipitate was obtained at pH 4.55, and this amount is less than the theoretical by 0.5 mg. This is equivalent to 0.05 mg. of formaldehyde which failed to precipitate in approximately 300 ml. of solution containing a 10 per cent excess of dimedon, and corresponds to an error of only 2 per cent in the precipitation of as little as 3 mg. of formaldehyde.

A similar method was used to determine the optimum pH for the precipitation of acetaldehyde.

Ten-milliliter samples of a 1 gram per liter acetaldehyde solution were added to 250-ml. buffer mixtures containing 17.5 ml. of saturated dimedon solution (10 per cent excess), and the solutions were filtered after 24 hours. Samples of the filtrates were caught as before, but examination after several days' standing

FIGURE 1. INFLUENCE OF pH ON FORMALDEHYDE-DIMEDON PRECIPITATE

Table I. Determination of Formaldehyde

Method	Formaldehyde Found *G./10 ml.*
Reduced silver	0.01075
Excess silver	0.01096
Dimedon, sample 1	0.01090
Sample 2	0.01084

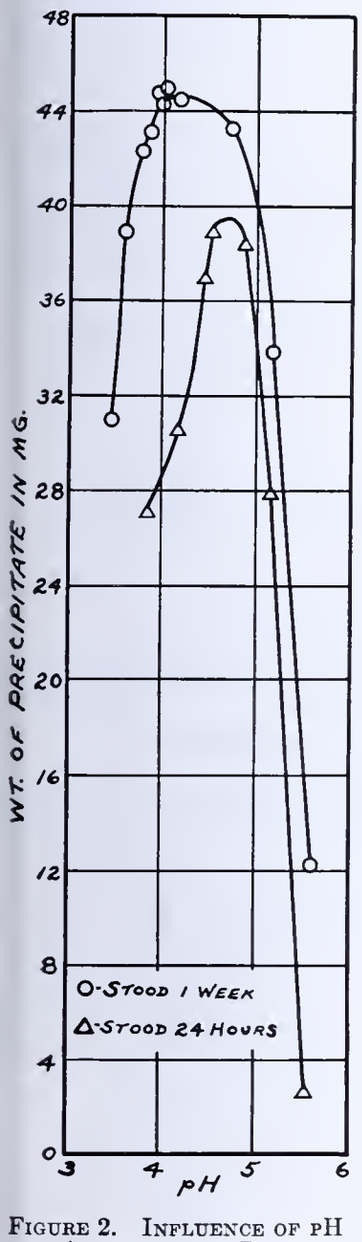


Figure 2. Influence of pH on Acetaldehyde-Dimedon Precipitate

showed that precipitation had been incomplete at the time of filtering. It was concluded that the rate of precipitation of acetaldehyde is slower than that of formaldehyde, and this was proved by qualitative tests. A new series of buffers was prepared and acetaldehyde was precipitated as before, except that the solutions were shaken several times a day and allowed to stand a week before being filtered. The precipitation was found to be complete in these solutions. Figure 2 shows the results of these investigations with acetaldehyde.

The solution from which 10-ml. samples were taken for these determinations was standardized by the method of Ripper (*4*) and found to contain 0.9588 gram of acetaldehyde per liter. Each 10-ml. sample should theoretically have yielded 0.0667 gram of precipitate. The greatest precipitation, at pH 4.02, was less than the theoretical by 0.0221 gram, corresponding to 3.2 mg. of acetaldehyde which failed to precipitate in approximately 300 ml. of solution containing a 10 per cent excess of dimedon.

In the determination of the optimum pH for precipitation of propionaldehyde, the method was the same as for formaldehyde except that 13.3 ml. of saturated dimedon solution were used for a 10 per cent excess over the amount required for reaction with 10 ml. of a propionaldehyde solution of 1 gram per liter, and 5 days were allowed for complete precipitation. Qualitative tests showed that the rate of precipitation of propionaldehyde is slower than that of formaldehyde but greater than that of acetaldehyde. The precipitation was incomplete after standing 24 hours, but complete after 5 days. The results are plotted in Figure 3.

The solution from which the 10-ml. samples were taken for these determinations was also standardized by Ripper's method (*4*) and found to contain 0.8480 gram of propionaldehyde per liter. The theoretical yield from a 10-ml. sample is 0.0468 gram of precipitate. The greatest precipitation, at pH 4.72, was less than the theoretical by 0.0095 gram, corresponding to 1.7 mg. of propionaldehyde which failed to precipitate in approximately 300 ml. of solution containing a 10 per cent excess of dimedon.

The results in the determination of the optimum pH for precipitating acetaldehyde and propionaldehyde indicated that small amounts of these aldehydes would not introduce an error in the quantitative precipitation of formaldehyde under these conditions. The determination of formaldehyde was unaffected by the presence of 3.2 mg. of acetaldehyde in 300 ml. of solution, and the presence of 1.7 mg. of propionaldehyde gave results in error by only 0.05 mg.

Solubility

To determine directly the solubilities of the precipitates in the buffer, a solution was prepared at pH 4.6 and filtered, and 400 ml. were added to weighed amounts of each of the condensation products in 500-ml. Erlenmeyer flasks. The flasks were then stoppered and placed in a constant-temperature water bath at 25° ± 0.1° C. The solutions were allowed to remain in the bath for 4 days with occasional shaking, and were then filtered. The undissolved residues were washed with a little water and dried to constant weight at 60° C.

To find the effect of excess dimedon on the solubility of the precipitates, the above procedure was repeated, except that 10 ml. of saturated dimedon solution were added to each solution before placing them in the bath.

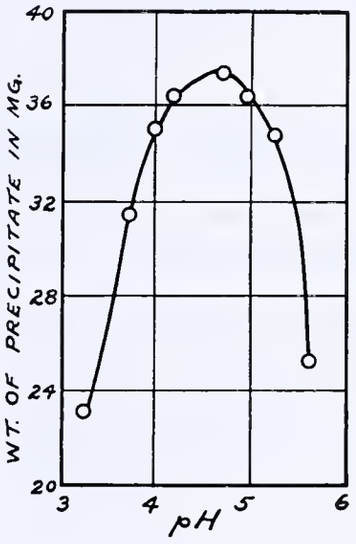


Figure 3. Influence of pH on Propionaldehyde-Dimedon Precipitate

The results are shown in Table II. Melting points of the undissolved residues showed that the compounds had undergone no change. It is clearly seen that a 10-mg. excess of dimedon per 100 ml. of buffer greatly decreases the solubility of the precipitates.

It was found that acid or alkaline solutions can be analyzed accurately for formaldehyde if first neutralized to the purple

Table II. Solubility Determinations

Dimedon Precipitate	Solvent	Weight of Sample *Gram*	Weight of Undissolved Residue *Gram*	Solubility *Mg./100 ml*
HCHO	400 ml. of buffer	0.0813	0.0808	0.1
$H_3C.CHO$	400 ml. of buffer	0.0820	0.0508	7.8
$H_3C.CH_2.CHO$	400 ml. of buffer	0.0956	0.0822	3.4
HCHO	400 ml. of buffer and 10 ml. of saturated dimedon solution	0.0788	0.0786	0.05
$H_3C.CHO$	400 ml. of buffer and 10 ml. of saturated dimedon solution	0.0362	0.0234	3.2
$H_3C.CH_2.CHO$	400 ml. of buffer and 10 ml. of saturated dimedon solution	0.0648	0.0624	0.6

color of bromophenol blue or to the yellow color of nitrazine indicator. The buffer (50 ml. of 1 *N* sodium acetate and 25 ml. of 1 *N* hydrochloric acid) to keep the solution at pH 4.6, and a 10 per cent excess of dimedon are then added and the solution is allowed to stand about 18 hours with occasional shaking before being filtered.

Stability to Heat

Results obtained in the determination of the stability of the formaldehyde-dimedon precipitate to heat showed that a 0.02-gram precipitate is at constant weight after heating 1 to 2 hours at 60° C., while heating for only 1 hour at 100° C. leads to a loss of approximately 0.5 mg. through sublimation.

Summary

Very accurate results can be obtained in the determination of formaldehyde with dimethylcyclohexanedione if the precipitation is carried out in a sodium acetate–hydrochloric acid buffer solution at pH 4.6. In such a solution containing a 10 per cent excess of reagent, only 0.05 mg. of formaldehyde remains unprecipitated in 300 ml. of solution and 1 mg. each of acetaldehyde and propionaldehyde do not precipitate in this same solution.

Twelve hours or longer should be allowed for the precipitation to take place, during which time the solution should be shaken occasionally. After being filtered through a sintered-glass crucible and washed with distilled water, the precipitate may be dried to constant weight in several hours at 60° C.

If an acid or alkaline solution of formaldehyde is to be analyzed, the sample is first neutralized to the purple color of bromophenol blue or to the yellow color of nitrazine indicator. The resulting solution is buffered at pH 4.6 and the precipitation carried out as before.

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Permanganate Titration of Thallous Salts

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SINCE Willm (*15*) first noted the necessity for chloride ion in titrations of monovalent thallium with potassium permanganate solutions, other investigators (*1*, *2*, *3*, *5*, *10*, *11*, *12*, *14*) have made the same observation under various conditions. They, however, obtained high results, the divergence increasing with decreasing concentrations of thallium. The work of three of these investigators is of particular interest.

Hawley (*5*), reporting his results in terms of a factor, gram of thallium per ml. of potassium permanganate, found that hot solutions, containing in a total volume of 60 ml. 4 ml. of hydrochloric acid (specific gravity 1.2) and about 0.1 gram of thallium gave nearly constant factors but that solutions containing smaller amounts of thallium gave lower factors. Similar results were obtained in cold solutions. Berry (*2*), following the method of Hawley, made a few titrations using an electrometric apparatus to determine the end point and obtained results 3 per cent high for a solution containing 0.0563 gram of thallium in 60 ml. He reported satisfactory results for a solution containing 0.253 gram of thallium in this volume. Swift and Garner (*14*) varied the concentration of hydrochloric acid from 0.5 to 1 *N* and the temperature from 55° to 90° C. but the end point was uncertain and the results were from 0.6 to 0.7 per cent high. They also observed little or no improvement upon addition of phosphoric acid alone or with manganous sulfate, addition of potassium chloride, or use of the iodine monochloride end point.

It seems to be generally believed that the high results obtained in the permanganate titration to a visual end point are due to oxidation of chloride ion. Since very little investigation of the electrometric end point has been reported, it seemed worth while to determine whether satisfactory results could be obtained by this method. In addition, titrations to a visual end point of solutions containing fluoride ion have been investigated.

Substances and Solutions

All reagents were of C. P. or equivalent grade. Stock solutions of thallous nitrate were standardized with concordant results by the chloroplatinate method (*4*) and by titration with standard potassium bromate (*7*, *16*). In some experiments, solutions of thallous sulfate similarly standardized were used. Aged potassium permanganate solutions, approximately 0.025 *N*, were standardized by means of National Bureau of Standards sodium oxalate.

Preliminary Experiments

A series of titrations making use of the visual end point was carried out at 65° to 70° C. on solutions 0.8 *N* in hydrochloric acid containing from 0.01541 to 0.1233 gram of thallium in a volume of 60 ml. The results were high by only 0.2 to 0.3 per cent in the stronger solutions but the per cent error became greater in the more dilute solutions, reaching a value of over 4 per cent in the most dilute solution studied. The end points faded rapidly. A similar series carried out at room temperature exhibited errors due to overtitration ranging from 2 to 10 per cent. These results are in agreement with those previously mentioned (*2*, *5*, *14*).

Titration to Electrometric End Point

The results of a series of electrometric titrations, using a bright platinum wire and a saturated calomel half-cell as

Table I. Electrometric Titration in Hot 0.8 *N* Hydrochloric Acid Solution with Nitrogen Atmosphere

Thallium Taken	Thallium Found	Error
Gram	*Gram*	%
0.00783	0.00792	1.0
	0.00787	0.5
0.01565	0.01579	0.9
	0.01573	0.7
0.02348	0.02362	0.6
	0.02357	0.4
0.03131	0.03134	0.1
	0.03132	0.0
0.04696	0.04706	0.2
	0.04717	0.4
0.06262	0.06238	−0.4
	0.06235	−0.4
0.07827	0.07805	−0.3
	0.07811	−0.2
0.09392	0.09375	−0.2
	0.09367	−0.3
0.1096	0.1096	0.0
	0.1095	−0.1
0.1252	0.1251	−0.1
	0.1250	−0.2

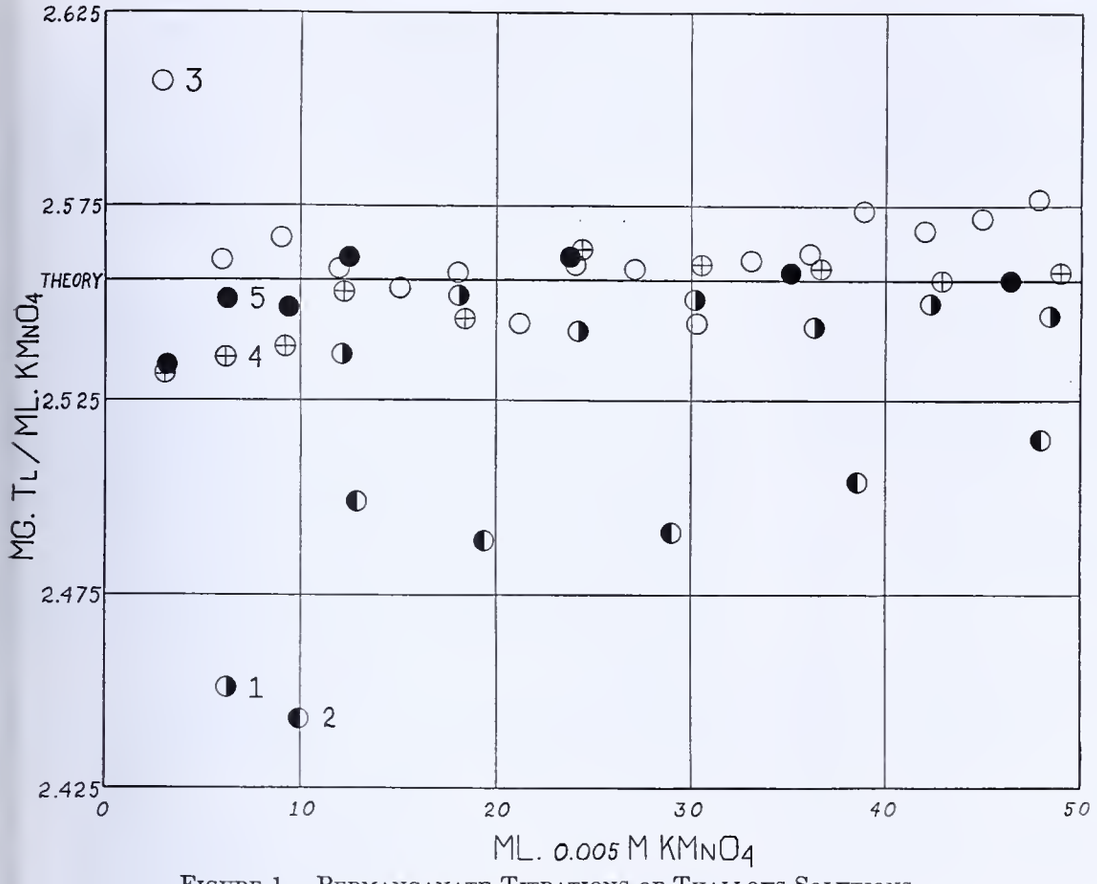

FIGURE 1. PERMANGANATE TITRATIONS OF THALLOUS SOLUTIONS

1. Hot solution with visual end point
2. Cold solution with visual end point
3. Hot solution with electrometric end point
4. Hot solution in nitrogen atmosphere with electrometric end point
5. Cold solution in presence of fluoride with visual end point

TABLE II. VISUAL TITRATION IN COLD SOLUTION 0.8 *N* IN BOTH SODIUM FLUORIDE AND HYDROCHLORIC ACID

Thallium Taken	Thallium Found	Error
Gram	*Gram*	%
0.00641	0.00648	1.1
	0.00644	0.5
0.01282	0.01287	0.4
	0.01280	−0.2
0.01922	0.01928	0.3
	0.01924	0.1
0.02563	0.02558	−0.2
	0.02554	−0.4
0.04870	0.04856	−0.3
	0.04856	−0.3
0.07177	0.07165	−0.2
	0.07173	−0.1
0.09483	0.09481	0.0
	0.09471	−0.1

electrodes, at 65° to 70° C. in 0.8 *N* hydrochloric acid solution indicated that detectable amounts of thallium were oxidized by atmospheric oxygen in the time required for these titrations. As a check on this point the loss of thallous thallium in warm hydrochloric acid solutions in beakers open to the atmosphere was shown, by the chloroplatinate method, to be as much as 4 per cent in 1 hour. With the elimination of this factor by constantly blowing a stream of nitrogen over the surface of the solution while it was being heated and during the titration, very satisfactory results were obtained. The solutions were all 0.8 *N* in hydrochloric acid and contained from 0.00783 to 0.1252 gram of thallium in a volume of 60 ml. Titrations to a visual end point with a nitrogen atmosphere showed no improvement over those conducted in air. The results of titrations to an electrometric end point are given in Table I.

The serious overtitration in the dilute solution range found with the visual end point is nearly eliminated. Further experiments indicated a very slow rate of attainment of equilibrium at room temperature and that 0.8 *N* was nearly the optimum concentration of hydrochloric acid for the warm solutions.

Titration in Presence of Fluoride Ion

Solutions 0.8 *N* in hydrochloric acid and containing an equivalent amount of sodium fluoride were titrated to the visual end point at room temperature. A faintly brown coloration appeared as titration progressed but the permanganate end point was clearly visible and the results were satisfactory, as shown in Table II. In the calculation of these results, account has been taken of the fact that the equivalent strength of permanganate in these titrations is 0.8 its normality as standardized, since permanganate ion is reduced only to the trivalent state in the presence of fluoride ion. The substitution of an equivalent amount of potassium fluoride dihydrate for the sodium fluoride in another series of titrations yielded similar results.

Further experiments were performed with solutions containing about 0.04 gram of thallium in order to ascertain the effect of certain variations from this procedure on the results. It was found that replacing a portion of the hydrochloric acid with an equivalent amount of sulfuric acid slowed the rate of reduction of permanganate. The results, however, were not seriously affected until approximately equivalent amounts of the two acids were present, when the end point became very uncertain. With the acidity maintained at 0.8 *N*, reduction of the sodium fluoride content to about 0.2 *N* was possible. Below this, the results were low and dropped off rapidly with further reduction of the sodium fluoride strength. In another series, the hydrochloric acid content was increased from 0.8 to 2.8 *N* in steps of 0.4 *N* and for each acidity the sodium fluoride strength was varied from 0.8 to 2.0 *N* in steps of 0.2 *N*. Solutions of high acidity and low sodium fluoride content gave low results. Those in which the acidity was low and the sodium fluoride high tended to be turbid but were otherwise satisfactory.

Consideration of these effects suggested the use of solutions 1.2 *N* in hydrochloric acid and 1.2 *N* in sodium fluoride for the titrations. The results of a series containing 0.02569 to 0.07707 gram of thallium in 60 ml. of this solution are listed in Table III and are seen to be satisfactory.

The data of Tables I and II together with the results of some of the preliminary experiments have been represented graphically in Figure 1, which permits a ready comparison of these results with the theoretical value as well as with each other.

The nature of the potentiometric titrations for thallium solutions may be seen in Figure 2. Both solutions were 1.2 *N* in hydrochloric acid, the one containing in addition an

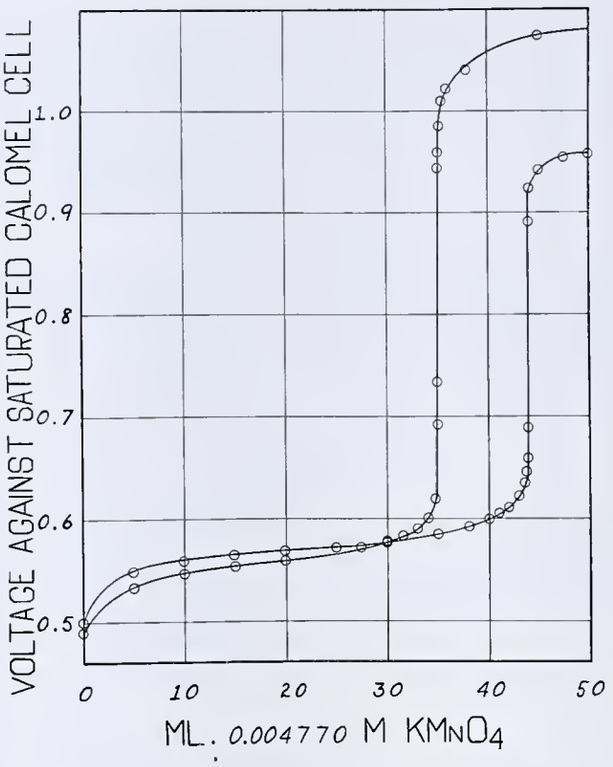

FIGURE 2. POTENTIOMETRIC TITRATIONS OF THALLOUS SOLUTIONS

Solutions had same thallium content. The curve to the right was obtained in the presence of fluoride.

equivalent amount of sodium fluoride. The titrations were carried out slowly at room temperature (27.5° C.).

It is interesting to compare the values of the potentials for these solutions when half oxidized, with the standard oxidation potential, −1.25 volts (*8*), for the system Tl^{+}–Tl^{+++} based on studies made in other mineral acid solutions. The value, referred to the normal hydrogen electrode, of about −0.799 volt obtained in the case of the solution free from fluoride ion indicates clearly the strong tendency of trivalent thallium to form complex chloride ions. This phenomenon causes also a very real dependence of the oxidation potential on the chloride-ion concentration of the solution, as was shown some time ago by Spencer and Abegg (*13*). These authors reported a value of −0.859 volt for a solution 0.105 *N* in hydrochloric acid and −0.828 volt for one 0.190 *N* in hydrochloric acid.

By analogy to ferric ion, the existence of a stable fluorothalliate complex ion might be expected. Although such an ion may be formed, the value of −0.815 volt obtained when thallium is half oxidized in the presence of fluoride ion indicates that its stability, if greater, is evidently not much more so than the corresponding chlorothalliate ion. It is not immediately evident why the potentials obtained in the presence of fluoride ion are larger than those in its absence. In view of the profound influence of the chloride-ion concentration on the Tl^{+}–Tl^{+++} potential, it is perhaps worth noting that the mean ion activity coefficient of hydrochloric acid is greater than that of sodium chloride in moderately strong solutions (*9*).

Recommended Procedure

Of the two satisfactory methods evolved in this work for the permanganate titration of thallous salts—namely, the electrometric titration in nitrogen atmosphere in hot 0.8 *N* hydrochloric acid solution and the visual titration in fluoride-ion solution at room temperature—the latter obviously is to be preferred because of its greater convenience.

The nature of the treatment of the solution prior to titration with potassium permanganate will, of course, depend upon the mineral from which the solution is obtained. In the monovalent state, thallium closely resembles the alkali metals in its analytical reactions; in the trivalent state it resembles many of the heavy metals. It is not infrequently necessary to effect a separation of thallium from other ions before proceeding with a quantitative estimation. Methods for the decomposition of thallium ores, for the separation of interfering ions, and for the preparation of a thallous solution are described by Hillebrand and Lundell (*6*).

Having prepared a solution in accordance with these considerations, to a total volume of approximately 60 ml. containing 6.0 ml. of hydrochloric acid (specific gravity 1.2) and thallous ion between 0.006 and 0.1 gram, add 3.0 grams of powdered sodium fluoride. (Instead of powdered sodium fluoride, a filtered solution containing 7.0 grams of potassium fluoride dihydrate may be used.) Titrate at room temperature with 0.005 molar potassium permanganate solution to a faint pink color which should persist for several minutes. A faintly brown coloration may appear as the titration progresses but the permanganate end point is clearly visible.

The normality of the permanganate solution, when used with fluoride ion, is four times the molarity. Thus, if the permanganate has been standardized by sodium oxalate, the normality so obtained should be multiplied by 0.8 to obtain the normality for titration in the presence of fluoride ion.

TABLE III. VISUAL TITRATION IN COLD SOLUTION 1.2 *N* IN BOTH SODIUM FLUORIDE AND HYDROCHLORIC ACID

Thallium Taken	Thallium Found	Error
Gram	*Gram*	%
0.02569	0.02574	0.2
	0.02572	0.1
0.03853	0.03857	0.1
	0.03855	0.1
0.05138	0.05141	0.1
	0.05139	0.0
0.06422	0.06409	−0.2
	0.06419	0.0
0.07707	0.07705	0.0
	0.07711	0.1

Summary

The work of previous investigators has been confirmed.

Electrometric titrations have been studied and found to be satisfactory in hot 0.8 *N* hydrochloric acid solutions under a nitrogen atmosphere.

A new method has been developed for the titration of thallous salts with permanganate in cold solutions by the use of fluoride ion.

Acknowledgments

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Measuring Oxidation of a Vegetable Oil

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IT IS WELL known that the peroxide number is not a valid measure of the extent to which oxidation has taken place in a vegetable oil. In view of the inadequacy of this standard method and the need for a more reliable method, the authors have been prompted to report the following studies.

A recently proposed method (*1*) for measuring the extent to which oxidation has taken place in a vegetable oil consists in determining the minimum concentration of freshly poured soap solution which will, after lapse of a constant period of time, prevent rapid spreading of a drop of the oil placed on the surface of the solution. Having already observed in this laboratory that a drop of benzene would not spread on a monomolecular film under considerable pressure and that a solution of Dreft quickly forms a surface film of considerable pressure, it was suspected that film pressure, and not film composition, determined whether or not benzene or a vegetable oil would spread on water. This proved to be the case for soybean oil, which is hydrophilic, and is presumably true for any liquid, a drop of which will spread appreciably on water. Washburn and Keim (*2*) have found it to be true for volatile organic liquids.

A very interesting and important behavior of soybean oil, when added to a water surface in an amount far exceeding that required to form a monomolecular film, is that some sections will be covered by a monomolecular film and other regions by a thick, plainly visible film, with very distinct and abrupt boundaries between these sections. The film pressure just at the point where drops will not spread when added to a surface covered by thin film, or just below which the drop will begin to spread, is readily determined by observation of the boundaries of the drops (or between areas with thin and thick layers), and thus constitutes a measure of the hydrophilic nature, or degree of oxidation, of an oil. The results are more easily reproducible if the drops are added to a region covered by a thin film.

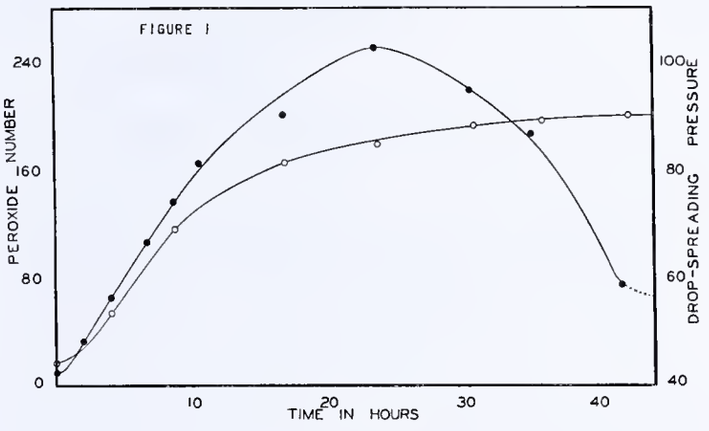
Experimental

All readings were taken on the Cenco Hydrophil balance, using distilled water as the substrate. The soybean oil was added to the water surface by means of a medicine dropper. After 2 drops had been added, the barrier was advanced to within about 10 cm. of the float. If the observed pressure was much greater than that necessary to prevent the third drop from spreading, the barrier was moved back and lifted for a moment to release some of the excess oil. If on again advancing the barrier to within about 10 cm. of the float the pressure was not within a reasonable range, the process was repeated or more oil was added, depending on whether the pressure was too great or too small. With a little experience one could readily approximate from the behavior of the pressure on adding the first 2 drops the pressure necessary to prevent the drop from spreading.

Table I. Oxidation of Soybean Oil

Time of Blowing of Oil *Hours*	Peroxide No.	Drop-Spreading Pressure[a]
00.00	9.44	44.3 44.6
4.00	65.5	53.3 53.7
8.50	135	65.8 65.9
16.75	197	81.0 81.1
23.75	249	83.9 84.3
30.25	218	87.5 87.2
35.50	185	87.7 87.9
42.75	72.7	88.5 88.6
48.00	48.4	89.0 88.9

[a] In scale units: to convert to dynes per cm. multiply by 0.2696.



With the pressure adjusted to a reasonable value, the third drop was added to a thin-film section. If the film pressure was sufficiently great, the drop would not spread and would remain indefinitely as a very thick lens (drop). The barrier was then moved back at a rate of about 0.5 cm. per second until the drop spread out and apparently lost its identity, as judged by the very distinct shadow which was cast on the bottom of the trough by the outer edge of the drop (lens). The pressure was always taken when this shadow became indistinct. This pressure is referred to as drop-spreading pressure.

It is significant that the thickness of the drop after it has lost its identity is about the same as that of the thick film and that, on moving the barrier still farther back, the pressure changes only very slowly, making it easy to obtain reproducible values. To test the applicability of this method air was blown through an edible soybean oil at a temperature of 100° C., samples being removed at intervals. Peroxide values in milliequivalents of peroxide per kilogram of oil and drop-spreading pressure values were obtained for each sample. Then the results were compared, as shown by the curves in Figure 1 and the numerical data in Table I.

It is at once apparent that the peroxide number rises to a maximum value in about 30 hours and then decreases to form a nearly symmetrical curve with two possible time values for each peroxide number; whereas the drop-spreading pressure rises continuously with time, though tapering off after about 15 hours.

Advantages

No weighing and no volumetric measurements are involved. The water surface does not have to be swept clean; the composition of the film apparently does not alter results; the barrier position before and after expansion of drop is of no appreciable significance; and after spreading of the drop the

pressure decreases very slowly as the film is expanded still farther, thus providing reproducible results. A medicine dropper gives a drop of convenient size; a drop may be added directly from the oil container; small variations in rate of expanding the film introduce no observable error; and the values do not rise to a maximum and then decrease (as peroxide numbers do). Presumably the method has wide applications, especially to the evaluation of lubricating oil addition agents which contain hydrophilic groups. It appears at present that the drop-spreading pressures are directly proportional to collapse pressures of monomolecular films of corresponding substances.

Summary

The measurement of the spreading pressure of a drop of liquid placed on a monomolecular film on the hydrophilic balance is a far more accurate evaluation of oxidation in a vegetable oil such as soybean oil or the presence of hydrophilic groups in any liquid than the familiar peroxide number. The evaluation of lubricating addition agents is an especially valuable application.

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Organic Acids in Plant Tissues

Modifications of Analytical Methods

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Modifications are described of previous methods to determine the total organic acidity, and the malic, citric, and oxalic acid content of dried plant tissues. These modifications permit complete analyses to be made on 0.5 gram or even on 0.1 gram of material, without sacrifice of accuracy and with greater convenience.

PLANT tissues, especially leaves and fleshy fruits, as a rule contain substantial proportions of relatively simple organic acids, chiefly polybasic hydroxy acids of the malic and citric acid type. Although much study has been given to the chemistry and physiology of these substances, surprisingly little of their exact functions in the metabolism of the plant is yet known. This is a field in which speculation still plays a dominating role, a consequence largely of the fact that satisfactory analytical methods for the more important of these substances have been developed only in recent years.

Methods for determining the total acidity of plant tissues due to organic acids (*6*), and malic (*7*), citric (*3*, *4*) and oxalic acid (*6*), together with nitric acid (*8*), have been described in previous papers from this laboratory. These methods depend on the observation that nitric acid and the organic acids can be quantitatively extracted by means of ether from the acidified dried tissue provided that an efficient extraction device is employed. Several years' experience in routine analyses of a variety of tissues has led to the introduction of a number of minor modifications that, taken together, materially increase the convenience and broaden the applicability of these methods. In the following description of these modifications, familiarity with the details of the earlier procedures will be assumed in order to save unnecessary repetition. The changes have chiefly to do with a reduction of scale so that complete analyses can be carried out upon 0.5 gram of dry tissue, or even on 0.1 gram instead of 2.0 grams without sacrifice of accuracy, and with an important simplification of the ether-extraction technique which eliminates the use of paper extraction thimbles.

Preparation of "Organic Acid Fraction"

The fresh tissue is prepared for analysis by being dried at 80° C. in a ventilated oven and is ground to a fine powder; of this 0.500 gram is thoroughly mixed in a small beaker with 1.0 ml. of 4 *N* sulfuric acid; 1.0 gram of asbestos is added and the whole is mixed with a rod until a homogeneous mass is obtained. The glass siphon cup of the extraction apparatus (Eimer & Amend catalog No. 30,754) is charged with angular quartz pebbles (about 5-mm. size) to a depth of about 1.5 cm., and over these is placed a folded pad consisting of several thicknesses of cotton gauze (cheesecloth) previously extracted with ether. The sample is then added, being packed evenly, and a second pad of gauze is used to wipe the last traces of tissue from beaker and glass rod and is gently pressed down above the sample in such a way as to cover it completely. The quantities of pebbles, gauze, and sample are such that the sample occupies approximately the middle third of the depth of the glass cup and sufficient gauze is used to prevent escape of the particles of tissue.

Extraction with specially purified ether is then conducted for about 16 hours (usually overnight) as previously described (*6*, *8*). The siphoning rate should be between 40 and 60 cycles per hour. (The completeness of the extraction depends on the number of times of siphoning. If extraction devices are used which siphon less frequently, the total extraction time must be correspondingly increased.) The ether in the extraction flask is then treated with 7 ml. of water and 5 ml. of 1 *N* sodium hydroxide (free from carbon dioxide) and the ether is evaporated in a water bath at 50° to 60° C. The aqueous solution is transferred to a 25-ml. flask and made to volume and is referred to hereafter as the "organic acid fraction".

Determination of Total Organic Acids

The changes introduced into the technique previously described for the titration of the total organic acids (*6*) are the substitution of a glass electrode for the quinhydrone electrode, the complete removal of carbon dioxide immediately before the titration, and the use of a more dilute nitric acid reagent. The advantages of the glass electrode on grounds of convenience do not need emphasis. All difficulties with platinum wire electrodes are eliminated and greater sensitivity is obtained.

No prescription with respect to the details of the apparatus is necessary; in this laboratory a Beckman glass electrode is used in conjunction with a Leeds & Northrup thermionic amplifier and student potentiometer with accessory equipment. The electrode is mounted, for convenience, on a discarded microscope stand, the rack and pinion of which provide for easily controlled introduction of the electrode into the solution. An agar bridge serves to make the connection with the standard calomel half-cell and has been found entirely satisfactory. The instru-

ment is calibrated against buffer solutions of known hydrogen-ion activity.

A 5-ml. aliquot of the organic acid fraction is transferred to a 30-ml. beaker with calibration marks at 20 and 25 ml. To this, 2 ml. of water, 3 drops of 0.04 per cent solution of bromocresol purple, and 1 ml. of 1 *N* nitric acid are added, the beaker is covered, and the contents are boiled gently for about a minute to expel carbon dioxide. The solution is cooled and neutralized with 1 *N* sodium hydroxide free from carbon dioxide, added from a fine-tipped buret, until a pale blue color is obtained. At this point, the glass electrode is introduced and the connections with the potentiometer system and half-cell are established. The potentiometer is set to the voltage that corresponds to a reaction of pH 8.0 as determined from the calibration. The solution should be slightly more acid than pH 8.0; if it is not, the reaction is adjusted with 0.1 *N* nitric acid, and 0.1 *N* sodium hydroxide is then added until the galvanometer deflects in the opposite direction. The galvanometer is finally brought to zero deflection by the dropwise addition of 0.05 *N* nitric acid. The potentiometer is next adjusted to the voltage that corresponds to pH 2.60, the acid buret is read, and acid is rapidly added until no deflection of the galvanometer is obtained. The solution is then diluted to the 20-ml. mark, and the titration is carefully completed. If more than 0.5 ml. of acid is required, it is necessary to dilute to the 25-ml. mark and again complete the titration by dropwise additions of the reagent.

A titration of water, completed at 20- and at 25-ml. total volume, is also made with the potentiometer set at pH 2.60, the result being subtracted from the titration value of the organic acid fraction. This blank value needs to be determined only occasionally; in this laboratory values of from 0.96 to 1.00 ml. were obtained during several months of routine titrations.

Two corrections are required before the titration data can be calculated in terms of total organic acidity. Oxalic acid must be separately determined, since this acid is titrated only to the extent of 50 per cent by the technique described, and sulfuric acid must also be determined, since the amount extracted by the ether under the present conditions, while small, is not entirely negligible if exact information is required.

To this end, the solution, after titration, is evaporated on a steam bath to about 12 ml., and is cooled and acidified with 0.5 ml. of 2 *N* hydrochloric acid; 50 to 100 mg. of asbestos are added and the solution is filtered with gentle suction through a Gooch crucible that contains a thin asbestos mat, into a 25-ml. wide-mouthed test tube. An apparatus constructed from a desiccator or a small bell jar with side tubulature provides for this filtration. The asbestos is washed with water until a volume of not more than 20 ml. of filtrate is obtained. To this, 6 *N* ammonium hydroxide is added until the indicator already present turns a faint blue color, and then 2 *N* acetic acid is added until a yellow color is produced, followed by 0.5 ml. of 5 per cent calcium acetate solution. After being thoroughly mixed, the solution is allowed to stand for at least 2 hours.

Filtration is more satisfactory than centrifugation (*2*) and is accomplished on a 1.5-ml. Gooch microcrucible that contains a disk of filter paper covered with a pad of fine asbestos specially prepared according to the directions of Kirk and Moberg (*1*). The test tube and the precipitate are washed with a saturated solution of calcium oxalate prepared according to Stanford and Wheatley (*9*) and the filtrate is reserved (see below). The test tube used for the precipitation is placed in the filtration apparatus under the crucible, and the calcium oxalate is dissolved by the careful addition of about 2 ml. of boiling 2 *N* sulfuric acid. This is stirred in the crucible with a fine rod and is then drawn through with gentle suction (see *2*). The treatment with hot sulfuric acid is repeated three or four times; the filtrate is then heated in a boiling water bath and titrated with 0.02 *N* potassium permanganate to an end point that is permanent for 10 to 15 seconds. One milliliter of the permanganate is equivalent to 0.09 mg. or to 0.02 milliequivalent of oxalic acid.

The sulfuric acid correction is ascertained from a sulfate determination in the filtrate from the calcium oxalate precipitate, and is calculated on the assumption that 3.6 per cent of the sulfuric acid extracted by the ether is titrated between the limits chosen for the organic acid titration. The filtrate is acidified to Congo red by the addition of 2 *N* hydrochloric acid and 5 ml. of 1 per cent barium chloride are added. The weight of the barium sulfate, obtained according to the usual technique, multiplied by 0.0063 gives the number of milliliters of 0.05 *N* nitric acid equivalent to the sulfuric acid titrated within the pH limits of the organic acid titration; this quantity is added to the water blank before calculating the total organic acids according to the formula given in the original paper (*6*). The magnitude of the sulfuric acid correction varies between 0.02 and 0.10 ml. of 0.05 *N* nitric acid reagent for a 5.0-ml. aliquot of the organic acid fraction.

Determination of Malic Acid

The modifications of the technique for the determination of malic acid have entirely to do with the details of the distillation of the oxidation product and with the filtration of the dinitrophenylhydrazine precipitate.

A suitable aliquot of the organic acid fraction, usually 5 ml., is prepared and oxidized as previously described (*7*). After removal of the pentabromoacetone with petroleum ether, the subaliquot (usually 25 ml.) is distilled directly, the addition of permanganate and of sodium sulfite being omitted. The original procedure occasionally gave low recoveries of malic acid since the addition of the sulfite reduced the acidity to a point where slight decomposition of the oxidation product might occur.

The receiving solution must not be allowed to boil vigorously during the distillation, owing to the possibility of loss of the volatile oxidation product. Accordingly, the technique is now as follows:

The subaliquot of the solution of the oxidation product, in a 300-ml. Kjeldahl flask, is diluted to about 50 ml. with water; if the aliquot is less than 25 ml., 1 or 2 drops of 18 *N* sulfuric acid are added, and the solution is distilled as previously described into a 250-ml. wide-mouthed Erlenmeyer flask that contains 10 ml. of filtered saturated dinitrophenylhydrazine solution and 20 ml. of water. The flask is supported in a small pan that contains about 250 ml. of cold water which is renewed for each distillation. This suffices to cool the distillate adequately. Distillation is continued until the residual volume is 5 ml.

The distillate is cooled to room temperature and filtered on a small sintered-glass funnel (Büchner type of fine porosity equivalent to Jena G4, treated if necessary with boiling alkali until a satisfactory filtration rate is obtained), and is dissolved in pyridine and treated as previously described. The calibration curve, from which the malic acid equivalent of the readings of the Pulfrich spectrophotometer is obtained, has been repeatedly checked within 5 per cent of the data given in the earlier paper. A Pulfrich spectrophotometer is not essential for the determination of the intensity of the color. There are several photoelectric devices available for which calibration curves can be established as described in the original paper (*7*) or, if necessary, a colorimeter can be used. In this case a standard solution of malic acid must be carried through the procedure in parallel with the unknown and a suitable quantity of the pyridine solution, found by trial and error, used for the final comparison.

Determination of Citric Acid

Only a slight change in the technique of the titration method for the halogen content of the pentabromoacetone obtained in the course of the method for citric acid (*4*) has been found necessary. It is essential to acidify the sodium sulfide solution that contains the decomposition products of the pentabromoacetone and to boil it to eliminate the hydrogen sulfide immediately. The solution may then be titrated for the halogen content when convenient.

A more satisfactory hydrogen peroxide reagent than that previously described may be prepared by pouring 16 to 18 ml. of 18 *N* sulfuric acid into 100 ml. of water, cooling the mixture to 5° C. in an ice bath, and adding about 10 grams of fresh sodium peroxide slowly so that the temperature does not rise above 30° C. The final solution should still be acid to Congo red paper. It is cooled to 5° C. and the fluid is decanted or centrifuged from the sodium sulfate. Prepared in this way the reagent contains between 2.5 and 3.5 per cent of hydrogen peroxide.

Analysis of 0.1-Gram Quantities of Tissue

Although the use of a 0.5-gram sample of dried tissue for the preparation of the organic acid fraction is more convenient and is recommended for routine use, occasions may arise when this quantity cannot be spared for the analysis. With attention to details of technique, it is possible to secure

Table I. Determinations of Organic Acids in Tobacco Leaf Tissues

(Data are given in milliequivalents per 100 grams of dry tissue)

Amount of Tissue Used for Preparation of Organic Acid Fraction	Total Organic Acids	Oxalic Acid	Malic Acid	Citric Acid
Grams	*M. e./100 grams*	*M. e./100 grams*	*M. e./100 grams*	*M. e./100 grams*
2.0	213 ± 9	40 ± 2	122 ± 6	30 ± 0.5
0.500	211 ± 6	39 ± 0.75	122 ± 3	30.3 ± 0.5
0.100	215 ± 3	39 ± 1	119 ± 2	30.3 ± 0.03

accurate analyses when only 0.100 gram of dried tissue is available.

The accurately weighed sample, in a watch glass, is treated with 0.3 ml. of 4 *N* sulfuric acid, placed in a depression in the heap of powder. Mixing is accomplished with a fine glass rod, and 0.2 gram of fine asbestos is then incorporated. The siphon cup of the extraction apparatus is filled with sufficient quartz pebbles so that the sample, on its pad of gauze, will occupy the middle of the height of the cup. Extraction is accomplished as already described. The ether is treated with 3 ml. of water and 1 ml. of 1 *N* sodium hydroxide solution, and is then evaporated. The aqueous solution is drained into the calibrated titration beaker for half a minute, and the residual traces are washed in with water in two successive 3-ml. portions, each being allowed to drain as before. The titration of the total organic acids is carried out as described, and the entire solution is used for the determination of the oxalic acid. The filtrate from the calcium oxalate is acidified with hydrochloric acid, and the sulfuric acid is determined as before. The filtrate from the barium sulfate is acidified with 3 ml. of 50 per cent sulfuric acid, is evaporated to 15 to 20 ml., and is used for the determination of the citric and malic acids.

Nitrate Nitrogen

The organic acid fraction contains the whole of the nitric acid present in the sample of plant tissue. The method of determining the nitric acid content, as originally described (*8*), involved the use of a colorimeter, and this modification has been studied by a group of collaborators and recommended to the Association of Official Agricultural Chemists as a tentative official method (*10*). The original description required the use of an aliquot of the organic acid fraction that contained the equivalent of 0.200 gram of dry tissue. Equally satisfactory results have been secured with the use of an aliquot that represents 0.100 gram of tissue (5 ml. of the organic acid fraction obtained as described above), and by reading the color of the nesslerized solution in a Pulfrich spectrophotometer, as described by Pucher, Vickery, and Leavenworth (*5*). Attention is merely called to this slight change in the interests of convenience to those laboratories equipped with this instrument. A photoelectric apparatus may doubtless be used equally well if calibrated correctly or, alternatively, the ammonia may be titrated with 0.02 *N* acid under appropriately standardized conditions.

Experimental

Many control analyses of pure acids and of previously analyzed tissues have been carried out to check the details of the modified techniques. A summary of the data secured is given in Table I, which shows mean values with the standard deviation of a single determination. In each case, four or more duplicate determinations are represented. It is clear that there is no choice between the use of 2 grams and 0.5 gram of tissue on the grounds of accuracy or precision; the smaller quantity is, however, somewhat more convenient to manage and is recommended for routine work. The use of 0.1 gram of tissue likewise gives satisfactory results but is recommended only when the supply of tissue is inadequate.

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Rapid Determination of Starch (Root) with Sodium Hypochlorite

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THE usual methods employed for the determination of starch are long and cumbersome and are unsuited for the control of manufacturing processes, especially in the starch industry. Rapid methods of analysis have been proposed from time to time and have found limited applicability. These methods depend upon complete dispersion of the starch into a colloidal solution which can be separated from extraneous matter (plant tissues) and then upon measuring it by precipitation and weighing, by combining with iodine which may be quantitatively determined, or by measuring the optical rotation of the starch solution with a polariscope or saccharimeter.

The principal difficulty encountered with the rapid methods is obtaining complete dispersion of the starch without undue hydrolysis or without solubilizing plant constituents other than starch. A very large list of chemical agents has been proposed for solubilizing starch for analytical work, but only a very few have found much favor. On certain materials hydrochloric acid (*6*) is satisfactory, but it requires carefully controlled conditions as to temperature and time of reaction which cannot always be adhered to in analyzing certain other plant materials because they filter with such extreme difficulty. Magnesium chloride (*2*) and calcium chloride (*5*) have been found fairly satisfactory in certain cases, but here solutions of high salt concentration are required and complete dispersion of the starch under the conditions specified, particularly in the analysis of sweet potato starch factory products, was not always obtained. Although dispersion with enzymes such as amylases might be considered ideal from a theoretical viewpoint, their application has also been found limited, at least under the conditions specified (*3*). The principal faults were that the solutions frequently filtered with difficulty and were often somewhat too turbid to be polarized with accuracy. Complete dispersion of the starch in the products being analyzed was not always obtained, especially in those products which had been treated with lime water (used in the factory process).

Since a rapid method for the determination of starch in

Table I. Effect of Varying Quantity of Sodium Hypochlorite on Polarization

(Solution contains 2.5 grams of starch)

5.25% NaOCl	Polarization	Clearness of Solution	Remarks
Ml.	° *V.*		
1	24.00	Good	No excess NaOCl in any sample after 5 minutes' boiling
2	24.05	Good	
3	24.00	Better	
4	24.00	Better	
5	23.85	Best	

sweet potato starch factory products was so desirable, a study was made of various agents which might be employed in solubilizing starch for its determination with a saccharimeter. Sodium hypochlorite, a strong oxidizing and bleaching agent known to have also a high power of converting raw starch even at room temperatures, was found to give excellent results. This agent is easily available and can be purchased under various trade names as a 5 to 5.25 per cent (by weight) solution in most grocery stores at relatively low cost; only small quantities of the active agent (2 to 15 ml. of commercial solution) are required per determination; the final solution filters easily, as a rule, yielding clear solutions that usually can be read in a polariscope without difficulty; and, with the simple procedure developed, the starch is completely dispersed (judging by the fact that the residue can be washed entirely free from starch with water). The principal disadvantage of the method is its apparent limitation to root starches. With the procedure outlined below, grain starches are apparently from 93 to 94 per cent converted; the unconverted fraction remains insoluble and is filtered off, yielding a solution whose polarization is, naturally, too low.

This observation suggests a possible rapid method for separating the so-called amylohemicellulose (*4*, *7*) from the amylopectin and amylose fractions of starch. It is likely that the portion remaining insoluble after the action of hypochlorite is amylohemicellulose because of its obvious presence in grain, but not in root, starches. This fraction, whatever it may prove to be, is, of course, convertible with malt diastase or hydrochloric acid, so that it is included in the determination of starch by the usual methods.

Preliminary Experiments in Developing Procedure

After it was determined that sodium hypochlorite would disperse starch satisfactorily in the presence of plant tissues—that is, without converting the starch to an objectionable degree—and yield a solution of starch which was clear and, hence, could be read in a polariscope, the details of a workable procedure had to be developed.

Although most starches are gelatinized at a temperature close to 70° C., it was found desirable actually to boil the samples of vegetable plant materials during the gelatinization period and during the reaction with hypochlorite; otherwise complete liberation of the starch from plant tissues might not be obtained. This procedure also ensured complete usage of the hypochlorite. Attempts were made to conduct the gelatinization and conversion operations in a 100-ml. volumetric flask in order to eliminate transferring, but because of erratic results and the tendency of some samples to foam when boiled, it seemed preferable to use a 400-ml. tall-form beaker. After treatment, the samples can be quantitatively transferred to a 100-ml. volumetric flask. However, gelatinization and hypochlorite conversion operations can be performed in a hot-water bath in cases where the starch is easily freed from the plant tissue and made soluble.

The amount of hypochlorite required to effect a desired degree of conversion of starch in the sample depends upon the material being analyzed. With pure or commercial starch, a suitable proportion of hypochlorite to starch is 1 ml. of 5.25 per cent solution per gram of starch in the sample. The tolerance is considerable, as may be noted from the tables, so that no undue precautions need to be observed in measuring the hypochlorite solution precisely.

In Table I are given the saccharimeter readings for 2.5 grams of sweet potato starch treated as follows:

Five 2.5-gram portions of starch were separately suspended in 50-ml. portions of water and treated with 1, 2, 3, 4, and 5 ml. of 5.25 per cent hypochlorite solution. These were heated to boiling and boiled for exactly 5 minutes; they were then transferred to 100-ml. volumetric flasks, cooled, made to the mark, filtered, and polarized in a 2-dm. tube with a saccharimeter.

Table II. Polarization of Starch Solutions

(Employing 3 ml. of NaOCl as a dispersing agent)

5.25% NaOCl	Starch in Sample	Polarization
Ml.	*Grams*	° *V.*
3	1	9.60
3	2	19.75
3	3	29.75
3	4	40.10

Table III. Polarization of Starch Solutions

(Prepared with a constant proportion of 1 ml. of 5.25% NaOCl per gram of starch)

5.25% NaOCl	Starch	Polarization	Remarks
Ml.	*Grams*	° *V.*	
1	1	9.95	All samples filtered clear, those of higher concentration increasingly slower.
2	2	19.80	
3	3	29.90	
4	4	39.95	

In the ratio of 5 ml. of the hypochlorite to 2.5 grams of starch, it is obvious that the reading is lowered. This effect is also apparent in Table II, which gives the readings obtained when employing 3 ml. of sodium hypochlorite solution with 1, 2, 3, and 4 grams of starch per 100 ml. following a similar procedure.

Under these conditions the polarization is not quite a linear function of the concentration. However, a linear relationship is shown by results, believed to be within experimental error, if a constant proportion of sodium hypochlorite to starch is employed, as may be observed from the data in Table III.

When, following the same technique as with commercial starch, 1 ml. of sodium hypochlorite solution was added to 5 grams of sweet potatoes containing approximately 1 gram of starch and boiled 5 minutes, etc., the starch was not completely dispersed. By varying the time of preboiling for gelatinizing the starch and softening the tissues, and by increasing the proportion of sodium hypochlorite to starch, conditions were established under which a limpid solution of starch was obtained with the tissues showing only traces of undigested starch, if any at all, when tested with iodine solution. The color reaction of the starch solution with iodine was purplish blue. These color reactions of starch solutions and of extracted tissue with iodine were the criteria used to establish the quantities of sodium hypochlorite solution to be used on different products. From the experiments it was evident that the tissues comprising the extraneous matter of the sample, in addition to the starch, were acted upon by the hypochlorite and that increasing quantities of hypochlorite must be used in analyzing products of a starch factory in which the ratio of starch to fiber is decreasing.

As with all other indirect methods of analysis, it was necessary to establish a factor for converting saccharimeter readings into starch. This is not a simple procedure because of uncertainties in the existing methods of starch analyses and one may rightfully question the accuracy of the factor to be suggested, because it is based upon the starch value obtained with malt diastase followed by acid hydrolysis. And again there arises the old question as to whether one should employ

Table IV. Relationship between Polarization and Starch Value of Sweet Potato Products Determined by Offical Malt-Diastase Method

Determination No.	Sweet Potatoes (5-Gram Samples) Starch by malt	Polarization[a]	Factor	Residual Pulp (5-Gram Samples) Starch by malt	Polarization[a]	Factor	Commercial Starch (2-Gram Samples) Starch by malt	Polarization[a]	Factor
	Grams	° *V.*		*Grams*	° *V.*		*Grams*	° *V.*	
1	1.2405	14.15	0.0877	1.3600	15.65	0.0873	1.2724[b]	14.20[b]	0.0899
2	1.3805	15.70	0.0879						
3	1.2175	13.85	0.0879	1.0745	12.85	0.0836	1.6628	18.90	0.0880
4	1.2395	14.10	0.0879						
5	1.1845	13.50	0.0877	1.0705	12.10	0.0885	1.6966	19.10	0.0888
6	1.1700	13.60	0.0860						
7	1.0810	12.30	0.0879	1.2070	13.70	0.0881	1.6684	18.90	0.0883
8	0.9915	11.40	0.0870						
9	1.0840	12.10	0.0896	1.1305	13.00	0.0870	1.6684	18.90	0.0883
10	0.9715	10.90	0.0891						
11	1.0355	11.60	0.0901	1.1075	12.40	0.0892	1.6616	19.00	0.0875
12	1.0240	11.60	0.0883						
13	1.0030	11.25	0.0891	0.8470	9.40	0.0901	1.6740	18.80	0.0890
14	1.1085	12.25	0.0905						
15	0.9890	11.10	0.0891	0.9340	10.35	0.0901	1.6628	18.85	0.0882
16	0.9495	10.75	0.0883						
17	1.0500	12.00	0.0875	....	...	....	1.6188	18.65	0.0868
18	0.9530	10.75	0.0887						
19	0.9610	10.90	0.0882	....	...	....	....	...	....
20	0.9050	10.15	0.0891						
Av.			0.0880			0.0880			0.0882

Grand average, 0.0880 gram per ° V.

[a] 2-dm. tube.

[b] 1.5 grams of commercial starch in this sample.

the usual equation of 0.9 × glucose = starch in this method to obtain the starch value or use a larger factor to compensate for the discrepancy between the determined value and the actual weight of starch corrected for known impurities. In this paper the writer used this equation and assumed the malt-diastase method would yield correct results, although in another paper (*1*) it is shown that serious errors may arise in its application to plant materials. The saccharimeter readings on solutions prepared from duplicate samples in which the starch was dispersed with sodium hypochlorite led to the conversion factor of 1° V. = 0.0880 gram of starch, which corresponds to a specific rotation of +193° for the starch in solution. This average factor held for the analysis of sweet potatoes and residual pulp, and was very close to that for commercial sweet potato starch. The results are summarized in Table IV.

Description of Method

It is, of course, essential that the sample employed for starch analysis be finely disintegrated. Sweet potatoes are most accurately and conveniently sampled by making a V-shaped cut in each of 10 or more of them with a sugar-beet sampling rasp and compositing. Residual pulp from the starch plant in the wet state is sufficiently fine to require no further disintegration. It is generally necessary, however, to grind dry pulp in order to reduce to a fine state the lumps and balls formed during drying operations. Commercial starch requires no further grinding unless it contains lumps in sufficient proportion to cause sampling errors. If grinding is necessary, care should be exercised to avoid breaking down the starch granules.

Weigh out a portion of the sample containing from 1 to 2 grams of starch and transfer to a 400-ml. tall-form beaker with about 100 ml. of water or of about 25 per cent alcohol solution in case of dried pulp containing gelatinized starch. After allowing mixture to digest at room temperature for about half an hour, add 10 ml. of a 10 per cent suspension of diatomaceous earth and transfer to a Gooch crucible fitted with an asbestos mat prepared in some standard manner. Wash the sample with about 100 ml. of water or dilute alcohol. Transfer the washed sample with asbestos mat quantitatively to the same beaker with from 50 to 75 ml. of water. Add a small quantity of sodium hypochlorite solution (consult Table V) and heat on a hot plate; boil the mixture 15 minutes, giving the beaker a rotating motion, or stirring occasionally to keep the insolubles suspended in the water the greater part of this period. Add the main portion of hypochlorite solution at the end of the gelatinization period and continue boiling for exactly 5 minutes, again agitating at times to rinse down the material which may collect on the sides of the beaker. Set aside for a few minutes and transfer while still warm to a 100-ml. volumetric flask. Cool quickly to room temperature; add about 5 drops of concentrated acetic acid (as an aid to clarification and filtration), make up to the mark with water; and add an extra quantity of water equal to the volume of the insoluble matter (chiefly asbestos and diatomaceous earth). In the tests reported, 1 ml. was taken as the correction volume, but each analyst should determine this correction for each type of material or change of procedure which might influence the value. Filter through fluted paper and read the rotation of the clear solution in a 2-dm. tube in a saccharimeter (or polarimeter). Calculate the starch from the reading in degrees Ventzke in accordance with the equation:

$$\% \text{ starch} = \frac{(^\circ \text{V.} \times 0.0880)\ 100}{\text{weight of sample taken}}$$

If the determination is carried out properly the starch solution, after the hypochlorite treatment, usually filters readily and is clear. However, a slight turbidity sometimes apparent seems to cause no appreciable difficulty in reading the solution because of its high dispersion. The starch solution should produce a purple to blue coloration with iodine; its reaction should be between 7 and 8 pH; and it should show no excess of hypochlorite when tested by adding a few drops of potassium iodide solution. If iodine is not liberated, giving a blue coloration in the presence of starch, hypochlorite is absent.

In Table V are given the pertinent conditions under which starch determinations are conducted, as applied in the analysis of sweet potato starch factory products. Although there has been no opportunity to analyze many other types of starchy plant materials, it is believed that the method is generally applicable to roots or tubers but not to grains or grain products.

Table V. Recommended Conditions for Analysis of Sweet Potato Starch Factory Products

Product	Weight of Sample	5.25% NaOCl Required Gelatinization	5.25% NaOCl Required Conversion	Time of Boiling Gelatinization	Time of Boiling Conversion
	Grams	*Ml.*	*Ml.*	*Min.*	*Min.*
Fresh potatoes	5.0	1	4	15	5
Potato flour	1.5	1	4	15	5
Wet pulp residue	5.0	1	14	15	5
Dry pulp residue	1.5	1	14	15	5
Starch (dry)	2.0	0.2	1.8	15	5

Acknowledgment

The writer wishes to express his appreciation to J. K. Phillips, Barberton, Ohio, formerly of this division, for assisting with the analyses reported in Table IV.

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Rapid Determination of Reducing Sugars

Extension of Forsee's Photocolorimetric Ferricyanide Method

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IN CONNECTION with a genetic study on the distribution of sugars in different varieties of watermelons it was necessary to devise a method whereby a large number of samples could be analyzed daily for both reducing and total sugars. Of the many sugar methods described in the literature, the clarification procedure and ferricyanide oxidation of Hassid (*7, 8*), as modified by Forsee (*4*) for rapid photocolorimetric determinations, seemed best adapted to the problem.

The principle of the Forsee technique is that the yellow color of alkaline ferricyanide, when heated with reducing sugars, diminishes in proportion to the quantity of sugar present, owing to the production of colorless ferrocyanide. This color decrease is then measured quantitatively with a photoelectric colorimeter, and a standard curve relating sugar concentrations to the colorimeter readings (or photometric densities) is prepared for routine use.

In the volumetric determination of reducing sugars by ferricyanide, a titration is conducted on either the residual reagent, as in the clinical iodometric methods of Hagedorn and Jensen (*5*) and its macromodification of Hanes (*6*), or the ferrocyanide formed, as in the ceric sulfate oxidation of Hassid (*7, 8*). A colorimetric modification for clinical micro work was introduced by Folin (*3*) who measured the ferrocyanide formed by conversion to Prussian blue. A more rapid technique based on the ferricyanide color itself was first used by Hawkins and Van Slyke (*9*), who measured the time required for the complete decolorization of the reagent. This procedure, however, requires continuous observation of each sample during an approximate 5-minute heating period. Hoffman (*10*) then described a photocolorimetric measurement of the decrease in yellow color and utilized this procedure for blood sugar determinations. It was applied to plant materials by Forsee (*4*).

The range of the present colorimetric ferricyanide methods was inconveniently narrow for this investigation: up to 0.2 mg. for the clinical Prussian blue and Hoffman methods, and 0.4 mg. for that of Forsee. The present modification makes possible the rapid estimation of up to 1.2 mg. of reducing sugar per aliquot oxidized: with two assistants it was possible to analyze daily about one hundred samples. It has been standardized for both glucose and invert sugar. No essential changes from Forsee's method have been made except as regards the volumes and concentrations of the reagents employed and an increase in the period of heating. A comparison of the ferricyanide oxidation to the standard copper methods has already been made by several investigators (*4, 7, 8*) and found very satisfactory for plant materials. Although the volumetric procedures permit far greater range—i. e., up to 10 mg. in Hassid's original procedure (*7*)—they are not so well adapted to the large number of determinations which can be rapidly and conveniently handled by the technique described below.

Apparatus and Reagents

The photoelectric colorimeter described by Evelyn (*2*) was used, equipped with a blue filter No. 420 (transmission limits 380 to 469 millimicrons). Six dozen 17.5 × 2.2 cm. (7 × 0.875 inch) absorption test tubes were selected which agreed to within 0.25 galvanometer unit. Complete analyses, including mixture of sugar aliquot with reagent, heating, and colorimeter reading, were conducted in the test tubes. In this manner rapid serial determinations were made without transfer of samples. Since the blue filter transmitted a weak light, the colorimeter lamp was always set to the bright position for taking readings.

A fresh solution of alkaline ferricyanide, exactly 0.05 per cent in potassium ferricyanide and approximately 1 per cent in sodium carbonate concentration, was prepared daily by pipetting 50 ml. of each of stock solutions A and B to a 2-liter volumetric flask and diluting to volume: Stock A, 2 per cent potassium ferricyanide–sodium carbonate prepared by dissolving 40.00 grams of reagent grade potassium ferricyanide and 40 grams of sodium carbonate monohydrate in water and diluting to 2 liters; stock B, 40 per cent sodium carbonate monohydrate. The reagents used for clarification by means of Hassid's (*7*) procedure were molar neutral lead acetate and 20 per cent disodium phosphate. For preparing the standard sugar curve, Bureau of Standards' samples of dextrose and sucrose were used.

The selection of 0.05 per cent ferricyanide concentration as the oxidizing reagent was determined by the necessity of obtaining a good spread in galvanometer readings, from approximately 10 to 90, as the sugar concentrations progressively increased. When the galvanometer was set at 100 with a tube of distilled water, a constant "center setting" (no absorption tube in the slot) of 74.75 was maintained. This ferricyanide concentration resulted in a consistent blank reading of 10.0. The galvanometer was read to the nearest quarter of a division.

Procedure for Calibrating Standard Sugar Curves

GLUCOSE. A solution containing 1 mg. per ml. was prepared by dissolving 2.000 grams of dextrose in 2000 ml. In order to obtain a series of a dozen 2-ml. aliquots containing from 0.1 to 1.2 mg., 5, 10, 15, etc., to 60 ml. were diluted to 100 ml. The 2 ml. aliquots were pipetted to the dry colorimeter test tubes, and 25.0 ml. of the reagent alkaline ferricyanide, 0.05 per cent potassium ferricyanide–1 per cent sodium carbonate, added from a 250-ml. dispensing buret. Blanks were included using 2 ml. of distilled water. The tubes were placed in a wire basket and set into a gently boiling steam-heated water bath in such manner that the contents were immersed to approximately two thirds of their depth. A piece of flat sheet metal was kept between the steam coils and the basket to prevent any uneven heating of tubes placed directly above the coils. Heating was maintained for exactly 10 minutes and the basket then quickly immersed in a beaker of water. A stream of tap water was continuously run into the beaker for approximately 10 minutes.

If care is observed to prevent splashing, especially during the heating period, the volume within the tubes remains sufficiently constant for the determination. The condensed water formed

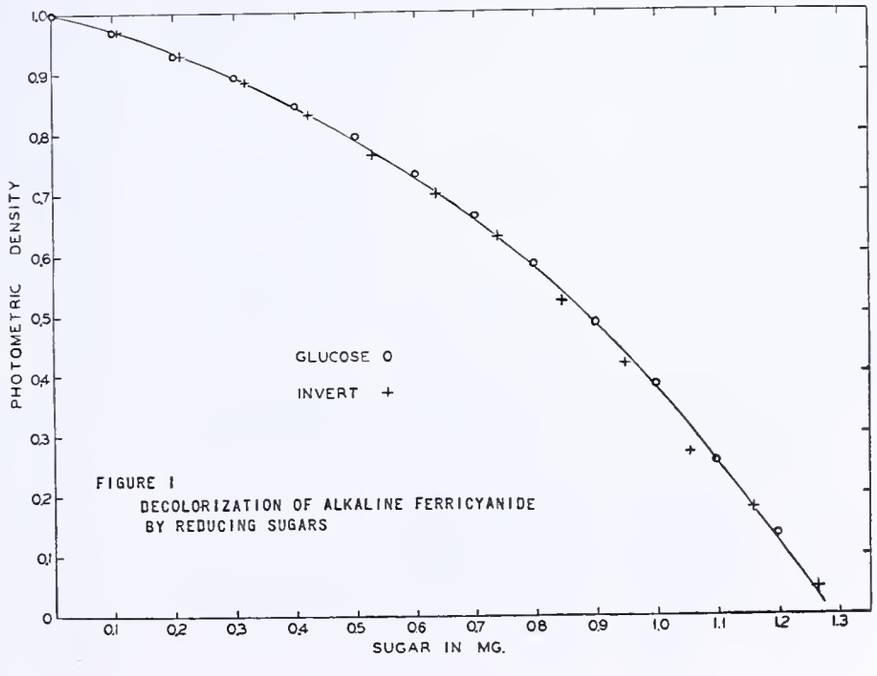

FIGURE 1
DECOLORIZATION OF ALKALINE FERRICYANIDE BY REDUCING SUGARS

Table I. Decolorization of Alkaline Ferricyanide by Reducing Sugars[a]

Glucose				Invert Sugar[b]			
Mg. per 2-cc. aliquot	Galvanometer Readings[c] Sept. 6	Sept. 7	Optical density (av.)[d]	Mg. per 2-cc. aliquot[e]	Galvanometer Readings[c] Sept. 6	Sept. 7	Optical density (av.)[d]
0.0	10.00 10.00	10.00 10.00	1.000	0.00	10.00 10.00	10.00 10.00	1.000
0.1	10.75 10.75	10.75 10.75	0.969	0.105	10.75 10.50	10.75 10.75	0.969
0.2	11.75 11.75	11.75 11.75	0.930	0.211	11.75 11.75	11.75 11.75	0.930
0.3	12.75 13.00	13.00 12.50	0.894	0.316	13.00 13.00	13.00 13.00	0.886
0.4	14.25 14.25	14.25 14.00	0.846	0.421	15.00 14.75	15.00 14.50	0.831
0.5	15.75 16.25	16.00 16.00	0.796	0.526	17.00 17.00	17.75 17.25	0.763
0.6	18.50 19.00	18.50 18.25	0.733	0.632	20.00 19.50	20.75 20.25	0.699
0.7	22.25 22.25	21.75 21.00	0.663	0.737	24.25 23.00	23.25 23.50	0.629
0.8	27.00 27.00	26.00 25.00	0.581	0.842	30.00 30.25	31.00 30.00	0.520
0.9	33.00 33.00	32.75 32.25	0.485	0.947	37.25 38.50	39.00 38.00	0.417
1.0	42.25 43.25	42.00 38.00	0.382	1.053	56.25 55.00	54.75 53.75	0.270
1.1	55.75 58.25	56.75 52.25	0.255	1.158	67.50 65.25	66.00 66.00	0.179
1.2	73.00 74.50	73.25 72.25	0.135	1.263	86.25 90.50	92.25 91.25	0.046

[a] 25 cc. of 0.05% $K_3Fe(CN)_6$–1% Na_2CO_3 heated with 2-cc. aliquots containing 0 to 1.2 mg. of sugar.
[b] On Sept. 7 inversion was conducted by official A. O. A. C. method (*1*); on Sept. 6 by immersion in boiling water for 10 minutes.
[c] Observed to nearest quarter division.
[d] Optical density = 2 − $\log_{10}$ galvanometer readings.
[e] Mg. of sucrose divided by 0.95 for conversion to invert sugar.

in the necks of the tubes was mixed with the contents by gentle shaking. The tubes were wiped dry with clean cheesecloth and read in the colorimeter. The instrument was set at 100 with distilled water and a constant center setting of 74.75 maintained. After cooling, the colors remained extremely stable. No change was detected up to 2 hours, and even when left overnight a maximum increase equivalent to only 1 per cent of the sugar present was observed. The tubes were therefore read whenever convenient within 2 hours.

Invert Sugar. A solution containing 10 mg. of sucrose per ml. was prepared by dissolving 2.000 grams of sucrose in 200 ml. Aliquots of 0.5, 1.0, 1.5, etc., to 6.0 ml. were transferred to 100-ml. Pyrex volumetric flasks and water was added to make a total volume of 5 to 6 ml. Ten milliliters of *N* hydrochloric acid were added and inversion was conducted in a bath at 70° C. according to the standard A. O. A. C. method (*10*) or by immersion in the boiling water bath for 10 minutes. The methods gave identical results (see Table I). The flasks were cooled in running water, 10 ml. of *N* sodium hydroxide were added, and the solution was diluted to volume. In this manner another concentration series of a dozen 2-ml. aliquots containing 0 to 1.2 mg. of sucrose was obtained. These values were converted to milligrams of invert sugar by dividing by the factor 0.95. The subsequent procedure was identical to that described above for glucose.

In Table I the data for both glucose and invert sugar, obtained in duplicate on successive days, are presented. For plotting the points on linear graph paper, the galvanometer readings, G, were converted to photometric density, L, using the equation $L = 2 - \log_{10}G$. (Galvanometer readings were not corrected for the slight deviations from true linearity of the relation between current and deflection. To make this correction, add $^1/_4$ unit from 9 to 25, $^1/_2$ unit from 26 to 38, and $^1/_4$ unit from 39 to 54 to the G readings in Table I.) The curve, so obtained is illustrated in Figure 1. It is interesting to note that the values for both invert sugar and glucose fall on the same curve, which during the course of the work, about 6 weeks, remained constant. A small deviation from the linearity of the theoretical log transmission–concentration relationship (Beer's law) was observed. For convenience in calculation, a table was prepared from the standard curve, by means of which any observed G reading was readily converted to milligrams of glucose or invert sugar.

Procedure for Plant Materials

The preparation of the samples for analysis as described by Hassid (*7, 8*) and Forsee (*4*) has proved entirely satisfactory. In the case of watermelon juice, a direct clarification with neutral lead acetate and disodium phosphate was conducted. Approximately 300-ml. samples of juice were prepared from the hearts of melons by reaming out with a spoon and gently pressing through two layers of cheesecloth. A clear juice was readily obtained by centrifuging and 10-ml. portions were transferred to 100-ml. volumetric flasks. Two milliliters of molar neutral lead acetate were added and the contents mixed. Five milliliters of 20 per cent disodium phosphate were added, mixed, and diluted to volume. The solutions were filtered through Whatman No. 12 fluted papers into dry screw-cap jars. Whenever it was necessary to preserve samples for subsequent analysis, several drops of a saturated solution of benzoic acid in toluene were added and the jars stored at 4° C.

For the determination of reducing sugars, 5-ml. portions of the filtrates were diluted to 100 ml. and 2-ml. aliquots were analyzed as described above for calibrating the glucose curve. For the determination of total sugars, 5-ml. portions were inverted and analyzed as described above for calibrating the invert sugar curve. By reference to the table the galvanometer readings were converted to milligrams of glucose and a factor of 10 directly converted the values to per cent sugar in the original juice (grams per 100 ml.).

As a test of the accuracy of the method, the recovery of various sugars added to watermelon juice samples was studied.

Three melons were selected at random and the juice was extracted and centrifuged as described. For each melon 10-ml. samples were analyzed in duplicate. A mixed sugar solution was prepared containing a final sugar concentration exactly 2.5 per cent in glucose, 2.5 per cent in fructose, and 5.0 per cent in sucrose. To the first two melon samples 5 ml. were added, or a supplement of 250 mg. of invert sugar and 250 mg. of sucrose (producing 263 mg. of invert sugar after hydrolysis) the samples from the third melon were supplemented with 10 ml., equivalent to 500 mg. of invert and 500 mg. of sucrose (526 mg. after inversion), respectively.

In Table II the results of these experiments are summarized. The recoveries were treated statistically as six pairs by subtracting the differences before and after supplementing with sugar. In the case of melon 3 the differences were divided by two, so that they could be combined with the results of the first two melons. The average recovery for 250 mg. of invert sugar added was 245, or about 98 per cent, with a standard error of 7 mg. for the mean of two determinations. The average recovery for 513 mg. of total sugar added was 509, or about 99 per cent; with a standard error of 10 mg. for the mean of two determinations.

Although the concentration range of the method described above represents a threefold extension of that of Forsee (*4*), for extremely dilute solutions, up to approximately 0.3 mg. of sugar per sample, the latter method affords a greater degree of precision. The calibration data were not given by Forsee, but a spread of 13 colorimeter scale divisions was reported for recoveries involving 0.1 to 0.3 mg. of glucose [(*4*), Table I]; whereas in the method described above (Table I) a spread of only 3 galvanometer divisions was observed in the range up to 0.3 mg. of glucose. (In a private communication, the author has been informed that a spread of 24.7 scale divisions was observed in the range 0 to 0.4 mg. of glucose.) If one reads the galvanometer to the nearest quarter division, then at the 0.2-mg. sugar level an error of approximately ±10 per cent results, which progressively increases with greater dilution; with increasing concentration, however, this error decreases in approximately the following manner: from 0.3 to 0.4 mg., ±7 to ±4 per cent; from 0.5 to 0.7 mg., ±2 to ±1 per cent; and from 0.8 to 1.2 mg., less than 1 per cent. In the range of higher concentration a variation of even several galvanometer divisions results in only a relatively small

Table II. Recovery of Sugars Added to Watermelon Juice

Melon No.	Reducing Sugars in 10 Cc. of Juice: Before Inversion: Galvanometer readings	Before Inversion: Mg. per 2-cc. aliquot	Before Inversion: Mg. per 10-cc. juice	After Inversion: Galvanometer readings	After Inversion: Mg. per 2-cc. aliquot	After Inversion: Mg per 10-cc. juice	Sugar Added to 10 Cc. of Juice: Invert Mg.	Sucrose as invert[b] Mg.	Reducing Sugars in 10 Cc. of Supplemental Juice: Before Inversion: Galvanometer readings	Before Inversion: Mg. per 2-cc. aliquot	Before Inversion: Mg. per 10-cc. sample	After Inversion: Galvanometer readings	After Inversion: Mg. per 1-cc. aliquot[e]	After Inversion: Mg. per 10-cc. sample	Recovery of[a] Invert sugar[c] Mg.	%	Total sugar[d] Mg.	%
1	17.00	0.528	528	20.75	0.661	661	250	263	24.75	0.763	763	18.75	0.594	1188	238	95	518	101
	17.00	0.528	528	20.75	0.661	661			25.00	0.768	768	18.50	0.585	1170	±3	±1	±9	±2
2	23.75	0.740	370	21.25	0.675	675	250	263	19.50	0.621	621	18.75	0.594	1188	248	99	511	100
	22.75	0.717[f]	359	20.75	0.661	661			19.00	0.603	603	18.50	0.585	1170	±14	±6	±16	±3
3	13.75	0.364	364	21.25	0.675	675	500	526	30.00	0.857	857	28.00	0.825	1650	500	100	996	97
	14.00	0.380	380	21.50	0.682	682			32.25	0.887	887	29.50	0.849	1698	±15	±5	±28	±3

[a] ± values represent maximum deviations from average recoveries.
[b] Mg. of sucrose added divided by 0.95 to convert to invert sugar formed after hydrolysis.
[c] Obtained by subtracting mg. of reducing sugars per 10 cc. of juice before inversion (column 4) from that observed after supplementing with known quantity of invert sugar (column 12).
[d] Obtained by subtracting mg. of reducing sugars per 10 cc. of juice after inversion (column 7) from that observed after supplementing with known quantity of invert sugar plus sucrose (column 15).
[e] Owing to high total sugar content, half of usual aliquot supplemented with 1 cc. water was oxidized.
[f] 10 cc. of clarified juice, rather than 5 cc. as usually taken, were diluted to 100 cc. before oxidation of 2-cc. aliquot.

error, due to the logarithmic relation between concentration and galvanometer deflection; at the 1.1-mg. glucose level, for example, the observed variation of 52.25 to 58.25 (the greatest galvanometer variation observed in Table I) results in an error of only ±2 per cent. In the routine analysis of large numbers of plant samples, usually involving great variation in sugar concentration, the sacrifice of a certain degree of precision in the extremely dilute range is well repaid by the saving of time and material consumed in reanalyzing samples requiring greater dilution.

Summary

A rapid photocolorimetric method for determining reducing sugars in aliquots containing up to 1.2 mg. has been described. The method is an extension of the procedure developed by Forsee for plant materials and represents a threefold increase in concentration range. The decrease in yellow color produced by heating reducing sugars with alkaline ferricyanide was measured with an Evelyn photoelectric colorimeter. Test-tube absorption cells were employed, permitting complete serial analyses without transfer. A reference curve for glucose and invert sugar has been prepared. The method was developed for rapidly analyzing large numbers of watermelons during a short harvesting period. Good recoveries were obtained when various sugars were added to watermelon juice.

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Contribution No. 7 from U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.

Decomposition Temperatures of Some Analytical Precipitates

Calcium Carbonate and Lead Sulfate

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ONE of the essential steps in many gravimetric analyses is the ignition of a precipitate either to remove a volatile impurity or to change the composition to a more desirable weighing form. The higher the temperature used, the less time will be required to fulfill the purposes of the ignition. However, since all compounds will decompose if heated to a high enough temperature, it is necessary to set a maximum temperature at which it is safe to ignite a precipitate without danger of decomposition.

In analytical chemistry we are concerned mainly with the decompositions which result in a loss of weight or, in other words, in which at least one of the decomposition products is a gas or a volatile substance. Only for those substances whose gaseous decomposition products are normal constituents of the atmosphere will it be possible to establish definite decomposition temperatures. For the class of substances whose gaseous decomposition products are not constituents of the atmosphere, it is not possible to state with exactness the ignition temperature below which no decomposition is possible. The statement of the maximum permissible ignition temperature will necessarily depend upon the conditions under which the ignition is carried out and upon the degree of accuracy desired in the determination.

The aim of these studies is to work out a satisfactory method of determining these dissociation pressures and of correlating the dissociation pressures with the maximum allowable ignition temperatures. The classes of precipitates which have been selected for study are the carbonates, sulfates, phosphates, and chromates. In deciding which individual compounds in each class to study, the authors have selected the list of weighing forms of various elements given by Lundell and Hoffman (*24*). The need for studying these decomposition temperatures becomes evident if we consider the great variation in the ignition temperatures for various precipitates

which are recommended by different investigators and in the standard texts on quantitative analysis.

In reviewing the various investigations pertaining to this subject, two types of results are found. The first are merely qualitative statements, such as "ignition at red heat produces partial decomposition" or "no decomposition results on heating with a blast lamp". Many of these statements were published before the advent of the thermocouple which made possible exact determination of high temperatures. The second type is more of a quantitative nature, either measurements of the dissociation pressures or loss in weight experiments.

One of the compounds which have been the most extensively and probably the most accurately studied is calcium carbonate, formed by the ignition of calcium oxalate at the proper temperature.

Willard and Boldyreff (*40*) state that it is advisable to carry out this ignition above 450° C. and that decomposition results above 550° C. The methods which have been used for this study include the determination of dissociation pressure (*8*, *16*, *20*, *23*, *26*, *30*, *34-36*, *38*, *41*), loss in weight (*6*, *15*), rate of gas evolution (*1*), gas saturation (*10*, *22*), "differential" method (*7*), emanation (*31*, *42*), and the use of a high-temperature vacuum balance (*4*, *32*, *37*). Probably the most accurate results are those of Johnston (*20*) who used a static method. His values are slightly lower than those of Pott or Zavriev (*41*) and he explained this discrepancy by the fact that in using a very small sample he was able to obtain a uniform temperature throughout the charge which cannot be done with a larger charge. This is a necessary precaution, since the pressure developed will be that in which the carbon dioxide is in equilibrium with the calcium carbonate at the lowest temperature.

The precipitate selected for this first investigation was lead sulfate.

Its decomposition was first mentioned by Berthier (*3*) who in 1822 stated that it decomposes slightly at white heat. Erdman (*11*) in 1854 found that this substance lost weight when heated in an open or loosely covered crucible. According to Boussingault (*5*) a loss of weight occurs on ignition at white heat. Percy (according to Mellor, *25*) in 1870 stated that lead sulfate was permanent at a low red heat. It was found by Bailey (*2*) that no decomposition results on igniting at 500° C. Friedrich and Blickle (*13*) gave the decomposition temperature as 850° C., while Hofman and Wanjukow (*17*) gave 637° C. as the temperature of the beginning of decomposition and 705° C. as the temperature at which dissociation becomes marked. Both these investigations were made by the thermal method. Mostovich (*27*) found that up to 800° C. lead sulfate does not change when heated in a current of dry air but at this temperature sulfur trioxide begins to be liberated. In 1906 Doeltz and Grauman (*9*) found no loss in weight between 700° and 800° C. and only a very slight loss at 900° C. but a big loss at 1000° C. Schenck and Rassbach (*33*) in 1908, Grahmann (*14*) in 1913, and Jaeger and Germs (*19*) in 1921 confirmed the decomposition at between 900° C. and 1000° C.

Method and Apparatus

For the determination of the dissociation pressures of the different precipitates at various temperatures it is desirable to have a single method which will be applicable to all. The vacuum static method of Johnston (*20*) and others, found so satisfactory for studying the decomposition pressures of carbonates, would not be equally satisfactory for studying sulfates and other substances whose gaseous products are not permanent gases at room temperature and would condense in the cooler part of the apparatus. Therefore the dynamic or gas saturation method was adopted. This method was first used for the determination of vapor pressures of solids by von Wartenberg (*39*) although Regnault (*29*) and others had used it earlier to determine the vapor pressure of liquids. This method consists of passing a measured volume of an inert gas over the heated substance, determining the amount of vapor in the gas, then from Dalton's law calculating the dissociation pressure. The method has also been used by Keppeler and d'Ans (*21*), Dutoit (*10*), Isambert (*18*), and Finkelstein (*12*) for determining the vapor pressures of various substances. Since these authors give insufficient details of their work and their results are not in agreement with those obtained by other methods, this method was first tried on calcium carbonate, as the dissociation pressures of this substance are probably the best known of any materials.

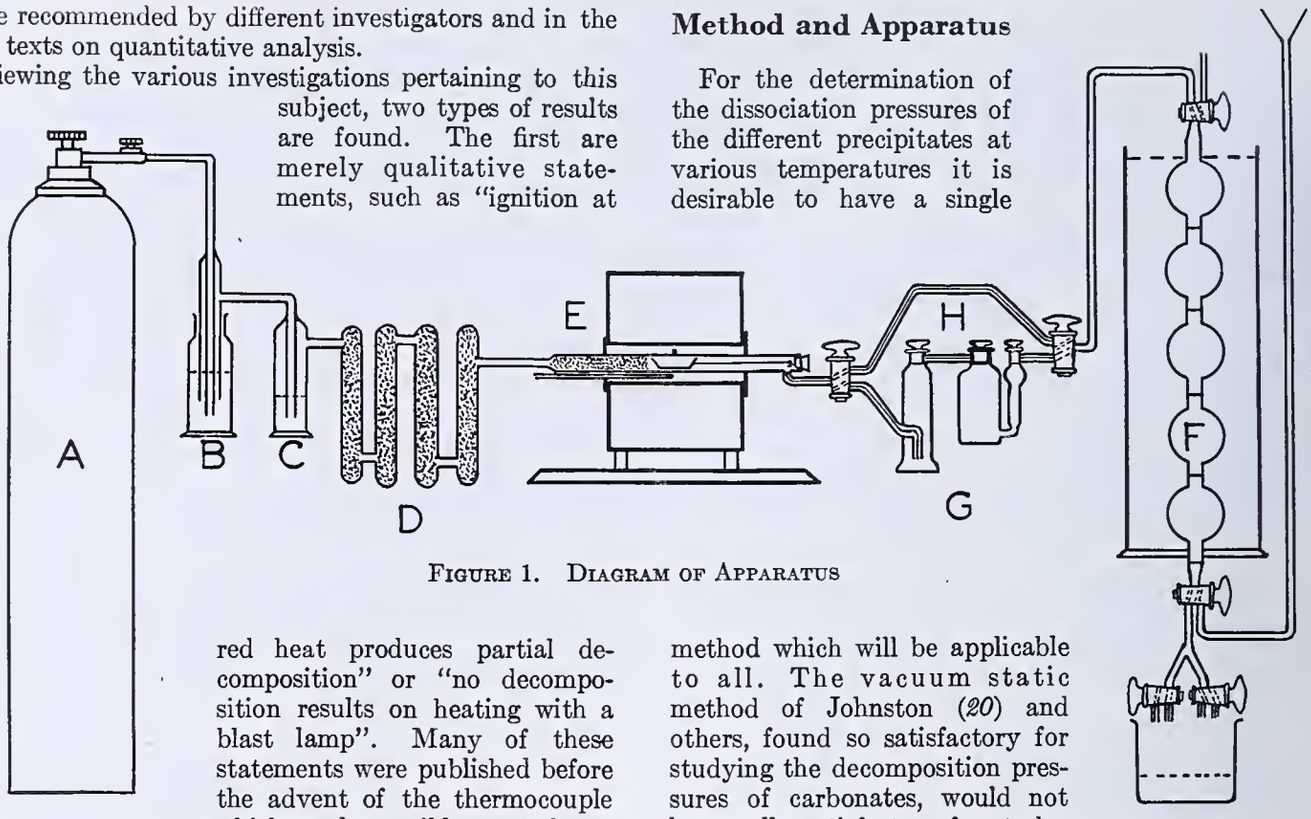

FIGURE 1. DIAGRAM OF APPARATUS

The apparatus used is shown in Figure 1. The nitrogen, which was used as the inert gas, passes from the cylinder, *A*, to a pressure regulator, *B*, through a bubble counter, *C*, and then through a series of tubes, *D*, filled with Ascarite and Dehydrite to remove all carbon dioxide and water. From these tubes it passes to the reaction tube, *E*, a microcombustion tube with a side arm, made of Pyrex or transparent quartz, depending upon the temperature being used. The front part of the tube is filled with small pieces of quartz which serve as baffles, making certain that the nitrogen is heated to the temperature of the sample. Following the quartz granules is the platinum boat containing the sample. The boat is 5 cm. long with the other dimensions such that it will just fit inside the combustion tube.

The combustion tube is heated by an electric furnace, *E*. From the combustion tube the nitrogen passes to the gas buret, *F*, going through either the absorption train, *G*, or the by-pass, *H*. The absorption train used with calcium carbonate consisted of a Stetser-Norton absorption bulb filled with Dehydrite for removing any water and a Wesson absorption bulb filled with Ascarite and Dehydrite. In several experiments Pregl micro absorption tubes were used. In all cases the microprocedure of wiping with moist flannel and chamois was used and a similar tube was used as a tare in weighing. The by-pass was used to permit flushing after introduction of the sample and to make it unnecessary to stop the flow of gas between the flushing and the beginning of the experiment.

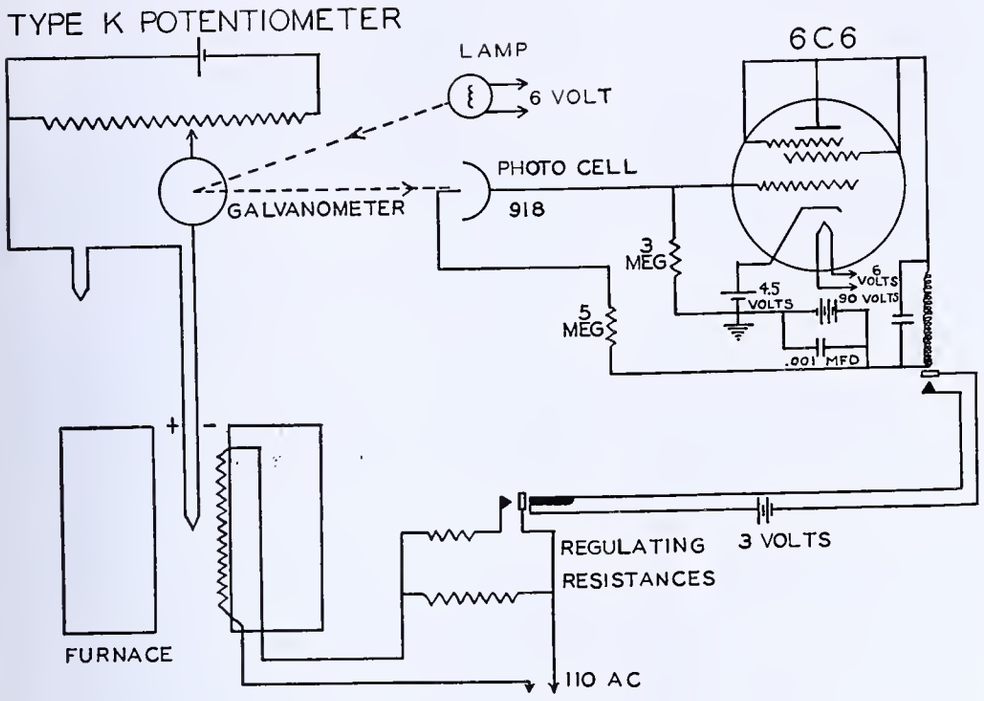

FIGURE 2. DIAGRAM OF REGULATOR

During preliminary runs, it was noted that the temperature of the furnace varied as much as 20° to 25° C. during a day, owing to variations in the line voltage. The furnace was therefore equipped with a temperature regulator with which it was possible to keep the temperature constant to within ±1.0° C. for any length of time. The regulator is shown in Figure 2. It was operated by a chromel-alumel thermocouple, the hot junction of which was placed outside the reaction tube directly beneath the boat containing the sample. The thermocouple was connected in the usual manner to a Leeds & Northrup Type K potentiometer, balance being indicated by a Leeds & Northrup Type R No. E reflecting wall galvanometer. The beam of light reflected from the galvanometer struck a photoelectric cell which, by means of amplification and two relays, switched on an auxiliary heating current. By adjusting the main heating current so that it was not quite sufficient to maintain the desired temperature, and by having the photoelectric cell in such a position that as the temperature fell the light beam struck the cell and turned on the auxiliary current, it was possible to keep the temperature practically constant. The proper key of the potentiometer was kept permanently depressed by a clamp.

The operating current was supplied by a 3-cell, 2-volt storage battery and was adjusted frequently with a standard cell. The cold junction was kept at room temperature, adjusting for any changes. The temperature readings of the chromel-alumel thermocouple were calibrated by a platinum, platinum-rhodium thermocouple, calibrated by the National Bureau of Standards, the junction of which was placed in the reaction tube in the same position that the platinum boat occupied in the experiment. The temperatures recorded in all the experiments are believed to be accurate within ±2.0° C. It was found that a space about 4 cm. long in the tube was at the same temperature and for a distance of 1 cm. on each side of this space it was not more than 1° C. lower. Therefore, in all experiments, the entire contents of the boat were within 1° C. of the same temperature.

Decomposition of Calcium Carbonate

Pure calcium carbonate was prepared from Baker and Adamson reagent grade calcium carbonate by double precipitation as the oxalate, drying, and then igniting for 12 hours in an electric muffle at 500° C. This temperature converts (*40*) all the oxalate to carbonate. The calcium carbonate was pulverized in an agate mortar and kept in a desiccator. Spectroscopic analysis of this calcium carbonate showed that it contained less than 0.01 per cent of magnesium and no appreciable amounts of any other impurity.

The platinum boat containing 0.5 gram of calcium carbonate was placed in the center of the reaction tube where the temperature was constant to within ±1° C. After replacing the stopper in the mouth of the tube, the flow of nitrogen through the tube and the by-pass was started by opening the stopcock at the bottom of the buret. Generally 500 ml. were used for flushing and increasing this volume seemed to have no effect. It is only necessary to flush sufficiently to remove all air and to give time enough for the sample to begin to dissociate and to saturate the gas with carbon dioxide. When the level of the water in the buret reached the top graduation, the stopcocks were turned to pass the gas through the absorption train. After the desired volume of gas had been aspirated through the system, the flow of water from the buret was stopped and the absorption train disconnected. In many cases several runs were made with the same sample by merely refilling the buret to the top graduation, inserting another absorption train, and omitting any flushing.

At the end of the run the temperature of the nitrogen was noted, the barometric pressure was read, and corrections for gravity, temperature, and capillary depression were applied, and the time required to carry out the run was recorded. The weighings were made as previously described. Any changes in barometric pressure during the experiment were neglected. The pressure in the tube was found to be within 0.2 mm. of the barometric pressure. Since this difference in pressure would have no significant influence on the results, it was also neglected.

The results for the determination of the dissociation pressures at various temperatures are tabulated in Table I and are shown in Figure 3 by plotting the logarithm of the pressure against the reciprocal of the absolute temperature. Only the values obtained at the faster rates were used and for comparison the values obtained by Johnston (*20*) and Andrussow (*7*) have also been plotted on the same graph.

To show the effect of varying rates of gas flow on the dissociation pressures, a series of experiments at 636° C. with different rates was made. The results are shown in Table II and Figure 4. The results at the slowest rate at 640° C. were

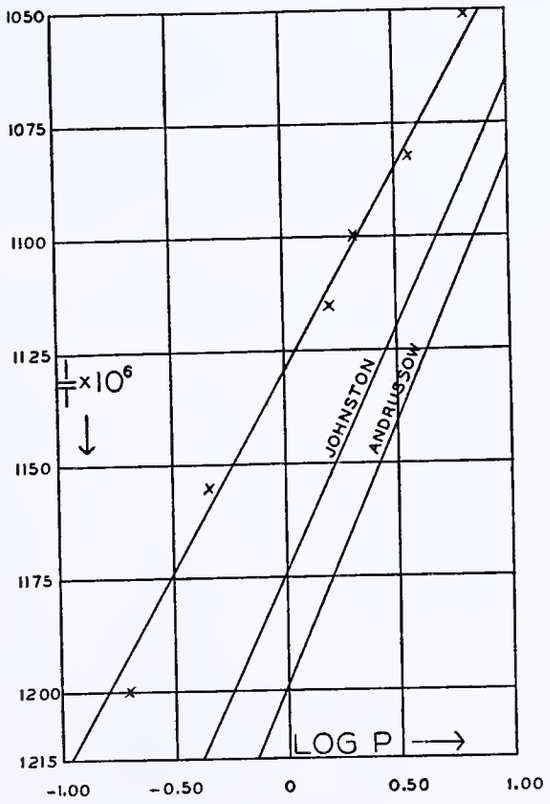

FIGURE 3. EFFECT OF TEMPERATURE

Table I. Dissociation Pressures

No.	Vol.	Temp.	Barometric Pressure	Volume at S. T. P.	Rate	Wt. of CO_2	p	log p	Temp.
	Ml.	*° C.*	*Mm. Hg*	*Ml.*	*Ml./min.*	*Gram*	*Mm.*		*° C.*
1	2031	22.8	727.3	1744	14	0.0004	0.08	−1.08	560
2	2031	23.0	727.3	1741	17	0.0010	0.21	−0.68	560
3	2031	23.3	733.2	1754	10	0.00155	0.33	−0.48	564
4	2031	24.0	737.3	1755	10	0.00092	0.20	−0.71	564
5	2031	22.3	728.7	1750	14	0.0018	0.38	−0.42	591
6	2031	22.8	724.2	1735	17	0.0026	0.55	−0.26	591
7	2031	23.5	723.3	1736	9	0.0021	0.45	−0.35	591
8	1811	21.8	733.2	1575	1.5	0.0065	1.54	0.19	615
9	1011	22.4	733.7	877	5.8	0.0035	1.49	0.17	615
10	2031	23.5	739.4	1765	17	0.0081	1.72	0.23	624
11	2031	23.8	739.0	1762	17	0.0085	1.81	0.26	624
12	2031	24.0	738.9	1759	23	0.0065	1.39	0.14	624
13	1011	21.0	723.0	870	10	0.0044	1.86	0.27	636
14	1011	20.8	724.1	872	10	0.0050	2.06	0.31	636
15	1011	20.8	725.1	873	10	0.0055	2.32	0.36	636
16	1011	20.7	727.9	877	10	0.0055	2.32	0.36	636
17	1256	20.7	727.9	1092	10	0.0058	1.97	0.29	636
18	1026	20.4	751.4	922	5.7	0.0059	2.45	0.39	636
19	1011	20.9	751.7	905	5.7	0.0045	1.87	0.27	636
20	1011	21.2	751.1	903	5.7	0.0052	2.20	0.34	636
21	1011	21.8	739.7	886	5.7	0.0040	1.70	0.23	636
22	1011	22.3	739.5	884	5.7	0.0050	2.13	0.33	636
23	1011	20.8	750.8	905	3.5	0.0065	2.74	0.44	636
24	1856	22.5	735.3	1613	1.7	0.0130	3.08	0.49	640
25	506	22.8	735.3	439	1.8	0.0040	3.40	0.53	640
26	506	22.0	734.5	440	1.5	0.0046	3.88	0.59	640
27	1624	21.6	741.9	1431	1.7	0.0132	3.47	0.54	640
28	575	20.5	739.4	508	1.5	0.0046	3.32	0.53	640
29	1011	24.0	746.2	885	18	0.0074	3.16	0.51	651
30	1011	24.0	746.2	885	18	0.0092	3.93	0.59	651
31	1011	24.3	745.4	883	13	0.0093	3.97	0.60	651
32	1011	24.7	745.2	881	13	0.0083	3.56	0.55	651
33	2031	22.0	732.5	1762	17	0.0320	6.70	0.83	679
34	2031	22.5	735.6	1765	17	0.0280	5.38	0.73	679
35	2031	22.5	737.8	1770	16	0.0293	6.16	0.79	679
36	2031	22.8	736.0	1773	14	0.0338	7.07	0.85	679
37	2031	24.5	740.6	1759	14	0.0324	6.87	0.83	679
38	2031	24.9	738.7	1750	12	0.0364	7.83	0.89	679
39	2031	25.0	738.7	1750	14	0.0288	6.20	0.79	679

corrected graphically to 636° C. In Figure 3 the rate of change of the logarithm of the pressure with the temperature is 0.0137 per degree, which agrees well with the corresponding values obtained from the curves of Johnston and Andrussow. Thus a change of 4° C. would change the logarithm of the pressure 0.05. The value for zero rate in Figure 4 is the equilibrium pressure determined by Johnston (*20*).

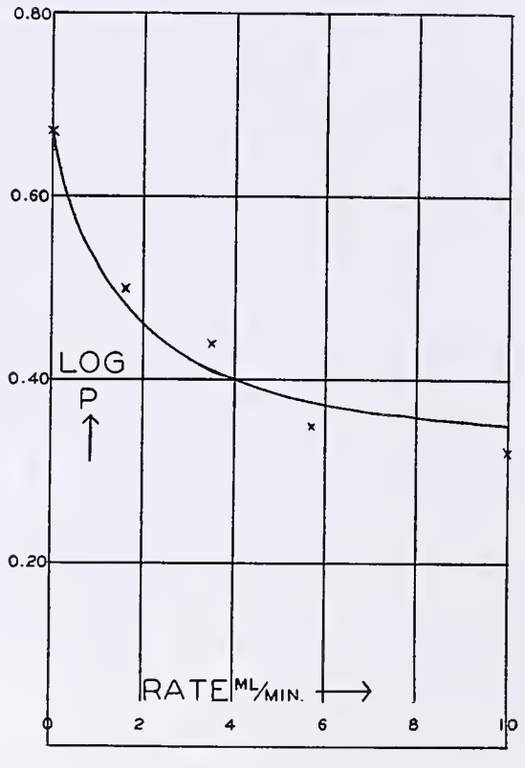

Figure 4. Effect of Rate of Gas Flow

It is evident from these results that the slower the rate, the nearer the pressures determined will approach the equilibrium values. This was shown by von Wartenberg (*39*) in his studies of the vapor pressure of the metals. He determined the vapor pressure at three different rates, and by plotting the pressure against the rate was able to make a straight-line extrapolation to zero rate. However, the rates he used were much faster, ranging from 11 to 100 ml. per minute. Such a procedure would not be justifiable in the authors' experiments, as shown by the rapid increase in the pressure at rates approaching the zero rate. Equation 1

$$y = \frac{0.043}{(x+1)^2} + \frac{2.637}{(x+1)} + 2 \qquad (1)$$

where x is the rate and y is the pressure, was found to fit this curve but seems to have no theoretical significance.

Decomposition of Lead Sulfate

Pure lead sulfate was prepared by adding, slowly and simultaneously, a solution of thrice recrystallized lead nitrate and dilute sulfuric acid to 5 liters of hot water, the lead nitrate being kept in slight excess. After thorough digestion, the lead sulfate was separated, washed, dried, and ignited at 500° C. Spectroscopic analysis showed that the lead sulfate contained no significant amount of impurity.

The decomposition of sulfates has two complicating factors. First, the decomposition product, sulfur trioxide, dissociates into sulfur dioxide and oxygen.

$$2SO_3 \longrightarrow 2SO_2 + O_2 \qquad (2)$$

Thus both oxides of sulfur must be absorbed and determined. Although it would be possible to determine these separately, it is not convenient and it would have no significance, as the reverse reaction

$$2SO_2 + O_2 \longrightarrow 2SO_3 \qquad (3)$$

may occur as the gases are cooled down on leaving the furnace. In one experiment at the highest temperature, after

Table II. Effect of Rate on Dissociation Pressures

(Temperature 636° C.)

Run No.	Rate *Ml./min.*	log p
13	10	0.27
14	10	0.31
15	10	0.36
16	10	0.36
17	10	0.29
		Av. 0.32
18	5.7	0.39
19	5.7	0.27
20	5.7	0.34
21	5.7	0.23
22	5.7	0.33
	Av. (of runs 18, 20, and 22)	0.35
23	3.5	0.44
24	1.7	0.44
25	1.8	0.48
26	1.5	0.54
27	1.7	0.49
28	1.5	0.48
	Av. (of runs 25, 26, 27, and 28)	0.50
	Johnston's value for equilibrium or zero rate	0.67

TABLE III. DECOMPOSITION OF LEAD SULFATE

No.	Volume of N_2	Temp. of N_2	Barometric Pressure	Volume at S.T.P.	Rate	Wt. of $BaSO_4$		p	log p	Temp.
	Ml.	*° C.*	*Mm. Hg*	*Ml.*	*Ml./min.*	*Gram*	*Liter*			*° C.*
1	2031	24.5	734.6	1745	4	0.00265	0.0015	0.11	−0.96	762
2	2031	23.5	740.5	1770	4	0.00264	0.0015	0.11	−0.96	762
3	1528	23.0	739.5	1333	1.4	0.00252	0.0019	0.14	−0.86	762
4	1518	23.5	737.2	1316	1.4	0.00268	0.0020	0.14	−0.86	762
5	2031	24.0	736.6	1755	1.3	0.00305	0.0017	0.12	−0.92	762
6	2031	24.8	734.8	1743	1.3	0.00277	0.0016	0.11	−0.96	762
7	1868	26.6	733.8	1535	1.1	0.00261	0.0017	0.12	−0.92	762
8	1668	27.0	733.0	1410	1.4	0.00220	0.0016	0.11	−0.96	762
				Averages at 762° C.			0.0017	0.12	−0.92	
9	1578	26.8	730.2	1330	1.5	0.00432	0.0032	0.23	−0.64	809
10	2031	26.2	730.4	1719	4	0.00512	0.0030	0.21	−0.68	809
11	1518	25.6	735.8	1298	1.4	0.00486	0.0037	0.27	−0.57	809
12	1908	25.0	736.0	1639	1.3	0.00531	0.0032	0.23	−0.64	809
13	2031	25.0	733.7	1740	4	0.00551	0.0032	0.22	−0.66	809
14	1618	25.0	728.1	1375	1.4	0.00486	0.0035	0.25	−0.61	809
				Averages at 809° C.			0.0032	0.23	−0.64	
16	3355	23.0	731.7	2897	2.5	0.02866	0.0099	0.69	−0.16	857
17	1956	22.5	743.7	1720	1.3	0.01850	0.0108	0.77	−0.11	857
18	1883	24.8	736.5	1620	1.6	0.01586	0.0098	0.69	−0.16	857
19	2031	25.0	734.5	1740	4	0.01851	0.0106	0.75	−0.12	857
20	1688	25.5	739.7	1453	1.4	0.01537	0.0106	0.75	−0.12	857
21	1518	26.0	734.2	1298	1.3	0.01233	0.0095	0.67	−0.17	857
22	1110	26.0	734.2	946	1.4	0.00991	0.0105	0.74	−0.13	857
23	1518	26.8	730.9	1282	1.3	0.01311	0.0102	0.72	−0.14	857
				Averages at 857° C.			0.0102	0.72	−0.14	
24	1478	27.3	730.6	1245	4	0.06625	0.0532	3.72	0.57	904
25	1728	27.2	726.6	1446	1.5	0.07772	0.0533	3.72	0.57	904
26	1981	26.4	726.9	1666	4	0.09160	0.0550	3.81	0.58	904
27	1518	25.0	732.1	1297	1.4	0.07002	0.0540	3.78	0.58	904
28	518	24.6	732.9	444	1.2	0.02232	0.0503	3.52	0.55	904
29	1201	24.5	735.8	1034	4	0.05999	0.0581	4.07	0.61	904
30	1036	23.0	742.1	908	1.2	0.04974	0.0548	3.89	0.59	904
31	1056	23.5	742.5	922	4	0.04999	0.0543	3.85	0.59	904
32	1011	23.5	742.2	882	4	0.04831	0.0548	3.89	0.59	904
33	1011	23.4	742.7	883	4	0.04795	0.0543	3.85	0.59	904
				Averages at 904° C.			0.0550	3.81	0.58	

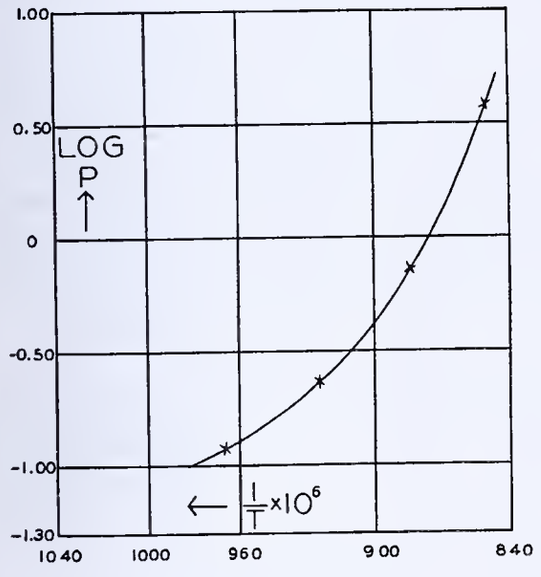

FIGURE 5

the alkali used to absorb the oxides of sulfur was made just neutral to methyl red and hydrogen peroxide was added to oxidize the sulfur dioxide, it required almost as much alkali as had been originally used to bring the solution back to neutral. This indicates that practically all of the sulfur trioxide was converted to sulfur dioxide and that the reverse reaction (Equation 3) is unimportant.

The other disturbing factor is the possibility of the sulfur trioxide condensing on the cooler parts of the apparatus before reaching the absorption flask. To prevent this the absorption flask was placed as near the reaction tube as conveniently possible. No visible signs of condensation were noted; but after a series of ten to fifteen runs, a small amount of sulfur trioxide fumes was visible on heating the part of the tube usually outside the furnace. Since this could not be prevented and as indicated previously, the amount of sulfur trioxide was probably much less than that of sulfur dioxide, this was neglected. However, it is a possible source of error.

The determinations were made the same as with calcium carbonate except for the absorption of the decomposition products. The oxides of sulfur were absorbed in dilute sodium hydroxide and determined gravimetrically, so no desiccant was needed. An excess of sodium hydroxide was used, about 10 to 15 ml. of 0.01 *N* at the lower temperatures and double this amount at the highest temperature. The alkali was placed in a 50-ml. Erlenmeyer flask, fitted with two delivery tubes. The tip of the inlet tube was drawn out to a capillary and dipped below the surface of the liquid. A second flask showed that all the oxides of sulfur were absorbed in this flask. The analysis for the oxides of sulfur was made by the standard microprocedure.

The results are given in Table III and a graph of the averages, plotting the logarithm of the pressure against the reciprocal of the absolute temperature, is shown in Figure 5. Only two rates were used and, as is evident from Table III, no significant differences are apparent at the two rates in this case.

Figure 6 is a graph of the same results showing the weight of sulfur trioxide per liter of nitrogen plotted against the centigrade temperature. It would seem that below 710° C. no appreciable loss in weight should occur when lead sulfate is heated in a muffle furnace, while at 730° C. a small but definite loss should occur. This prediction was tested by determining the loss in weight of lead sulfate on heating.

A platinum-wound muffle furnace (*28*) of about 700-ml. volume was used. The temperature regulator previously described was used and the temperature was measured with a platinum, platinum-rhodium thermocouple. The temperatures are believed to be accurate to within ±5° C.

Two samples of lead sulfate were weighed into small 8-ml. platinum crucibles and placed in the center of the muffle. One crucible was covered with a loose-fitting lid while the other was left uncovered. After heating the desired length of time, they were removed from the muffle, allowed to cool for 20 minutes in a desiccator, and weighed. The same samples were used repeatedly.

The results are given in Table IV. It can be seen that a small loss in weight occurred during the first 2 hours at 680° C. This is probably due to loss of moisture rather than decomposition, since further heating at this temperature and

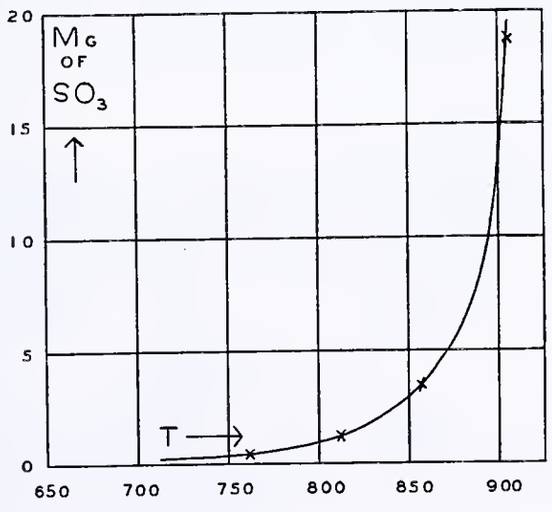

FIGURE 6

TABLE IV. LOSS IN WEIGHT OF LEAD SULFATE

Temp.	Time		Sample 1 (Covered). Original Weight, 1.5469 Mg.				Sample 2 (Uncovered). Original Weight, 1.1671 Mg.			
				Loss in Weight				Loss in Weight		
	Each ignition	Total at each temperature	Weight after each ignition	Loss in each ignition	Total at each temperature	Loss per hour (av.)	Weight after each ignition	Loss in each ignition	Total at each temperature	Loss per hour (av.)
° C.	*Hours*	*Hours*	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%
680	2	2	1.5465	0.4	0.4		1.1668	0.3	0.3	
	4.5	6.5	1.5465	0.0	0.4		1.1669	(0.1)	0.2	
	12	18.5	1.5464	0.1	0.5	0.0018	1.1668	0.1	0.3	0.0019
700	3	3	1.5463	0.1	0.1		1.1667	0.1	0.1	
	3	6	1.5463	0.0	0.1		1.1667	0.0	0.1	
	5	11	1.5463	0.0	0.1		1.1667	0.0	0.1	
	11	22	1.5463	0.0	0.1	0.0003	1.16665	0.05	0.15	0.0008
720	12	12	1.5463	0.0	0.0		1.1665	0.15	0.15	
	10.5	22.5	1.5462	0.1	0.1		1.1664	0.1	0.25	
	29	51.5	1.5462	0.0	0.1	0.0001	1.1660	0.4	0.65	0.0011
730	16	16	1.5460	0.2	0.2		1.1655	0.5	0.5	
	7	23	1.5460	0.0	0.2		1.1653	0.2	0.7	
	42	65	1.5453	0.7	0.9	0.0009	1.1635	1.8	2.5	0.0033
800	1	1	1.5449	0.4	0.4		1.1628	0.7	0.7	
	1	2	1.5449	0.0	0.4		1.1626	0.2	0.9	
	1	3	1.5447	0.2	0.6		1.1621	0.5	1.4	
	1	4	1.5445	0.2	0.8		1.1618	0.3	1.7	
	1	5	1.5444	0.1	0.9	0.019	1.1616	0.2	1.9	0.033

at 700° C. caused no further appreciable loss. At 720° C. the sample in the open crucible showed a slight loss in weight on each ignition while the weight of the sample in the covered crucible remained almost the same. At 730° C. the decomposition is slightly more marked, although heating for 1 or 2 hours at this temperature would not introduce a noticeable error when igniting samples as large as 1 gram. The loss in weight of 800° C. is appreciable in the course of 1 hour. It is of interest to note that both samples turned slightly yellow at the temperature at which the loss in weight became noticeable. To be certain that the loss in weight reported is not due to volatilization of the platinum, the weights of the crucibles before use were compared with those after all the ignitions were completed. The total loss in weight of the covered and uncovered crucibles after 162 hours' heating was 0.1 and 0.2 mg., respectively.

Summary

The dissociation of calcium carbonate has been studied by the gas saturation method of determining vapor pressures and the results have been compared with the values obtained by Johnston with a static method.

The effect of the rate of gas flow in this method has been studied and the results indicate that equilibrium values are not attainable at rates which are convenient to use. The slower the gas rate, the nearer the pressures approach equilibrium values.

Since this method more nearly duplicates the conditions in an actual ignition than the static method, the dissociation pressures obtained should be of more value in predicting the maximum possible ignition temperatures for analytical precipitates.

The dissociation pressures of lead sulfate at four temperatures have been determined.

A prediction of the temperature at which a noticeable loss in weight will occur when lead sulfate is ignited has been made and experiments to determine the loss in weight have borne out the prediction. Under the conditions described the loss in weight of lead sulfate due to decomposition first becomes appreciable at 730° C.

Acknowledgments

The authors are indebted to Drs. Lawrence and Curtiss of the Department of Mathematics for the derivation of Equation 1, and to Dr. Gartlein and Mr. Higinbotham of the Physics Department and Dr. MacMillan for the design of the regulator.

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A Precision Cryostat for the Range −35° to +25° C.

An Improved Electrical Circuit and a New Expansion Valve

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IN A PREVIOUS article (*1*), a description was given of a precision cryostat which continuously maintained temperatures to within a few thousandths of a degree within the range −35° to +25° C. Two important improvements in the system have since been made and are described herein.

The electrical circuit shown in the previous article (*1*) can be considered to consist of two main parts, the only connection between them being established through the grid circuits of the electron tubes; one part, containing the automatically controlled autotransformer (Variac), supplied modulated energy continuously to one heating coil immersed in the bath, called the steady heater, and the other part, containing the Thyratron tube, supplied on-off energy to a second heating coil, designated as the intermittent heater. This circuit had been so arranged in order to eliminate the undesirable effects of large thermoregulator current, interaction of grid currents, and failure of the Thyratron to "fire" if the voltage drop across it became too low. The use of a separate heating coil for the on-off energy input to the bath led to frosting inside the heating coil sheath, owing to the "breathing" effect, to the minute amount of energy being dissipated, and to the fact that the coil was energized but approximately half of the time. At times this frosting led to undesirable stray currents in the bath.

A simpler circuit with several advantageous features has been developed and is shown in Figure 1, the lettering being made to correspond to Figure 3 (*1*), and additional parts being assigned new designations.

Only one electron tube, the Thyratron, is employed, resulting in a simplified grid circuit and somewhat smaller grid currents. The modulator remains the same as before, as does the supply to the steady heater from the automatically controlled output of the Variac, but in the present circuit the intermittent energy input is superimposed upon the steady current, employing only one heating coil immersed in the bath. The resultant operation is somewhat more satisfactory than the previous circuit, the thermal lag in the intermittent heating is slightly decreased, and no frosting takes place in the heating coil sheath. The manner of intercoupling the Variac and the Thyratron is apparent from Figure 1, the small transformer, *P*, serving to induce a small additional current in the main circuit, *M*4-*H*1-*R*5-*E*. There is no danger that the voltage drop across the Thyratron will attain a low value, for it is not appreciably influenced by the main circuit. The proper voltage drop across *R*4 is secured by adjusting *R*8. Adjusting the taps at *X* on the secondary of transformer *P*, and if necessary changing the values of *R*6 and *R*7, the intermittent current may be made as small as desired. A very desirable characteristic of such a type of control, although not required for the present installation, is that by interchanging leads *Y* and *Z* on *P*, a decrease in the energy supplied to heater *H*1 is secured when Thyratron *T*2 passes current.

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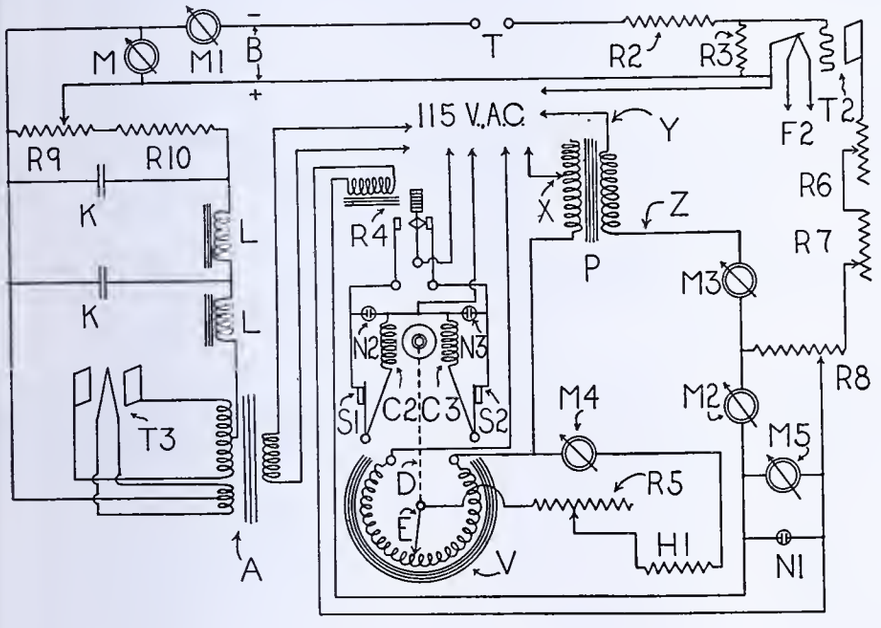

FIGURE 1. IMPROVED ELECTRICAL CONTROL CIRCUIT

A. Power supply transformer for rectifier
*T*3. Type 80 rectifier electron tube
L. Choke coils, 30 henries, 15-milliampere capacity
K. Capacitances, 4 mfd., 300 volts
*R*9. Variable resistor, 5000-ohm, 2-watt size
*R*10. 10,000-ohm fixed resistor, 2-watt size
M. Direct current voltmeter, 0 to 50 volts
*M*1. Direct current microammeter in thermoregulator circuit, 0 to 50 microamperes
B. Rectifier-filter output, 0 to 25 volts
T. Thermoregulator connections
*R*2. Resistor, 0.5 megohm
*R*3. Grid leak resistor, 2.0 megohms
*T*2. Type FG-57 Thyratron tube
*F*2. Heater connections for *T*2, 5.0 volts
*R*6. Variable resistor, 30 ohms, 3 amperes
*R*7. Variable resistor, 100 ohms, 1.5 amperes
*R*8. Variable resistor, 65 ohms, 2 amperes
*M*3. Ammeter, 0 to 3 amperes
P. 150-watt toy transformer, primary *YZ* in Thyratron circuit, secondary in Variac circuit
X. Variable selector switch on secondary of *P*
*R*4. Mechanical relay, 11,000 ohms, contact in position shown for cooling part of cycle when relay is not energized—that is, Thyratron not passing current
*C*2. Telechron stator coil, energized on heating part of cycle to rotate movable arm *E* on Variac clockwise, increasing amount of energy supplied to heater, *H*1
*C*3. Telechron stator coil, energized on cooling part of cycle to rotate *E* counterclockwise, decreasing amount of energy supplied to *H*1
*N*2. Neon glow lamp across leads to *C*2, 2 watts
*N*3. Neon glow lamp across leads to *C*3, 1 watt
*S*1, *S*2. Safety contact devices, normally closed as shown; a special arm on *E* prevents it from passing beyond end of normal path, by opening *S*1 or *S*2.
D. Mechanical coupling between Telechron rotor and *E*
E. Variable rotating contact switch on Variac
V. Variac, a variable autotransformer
*M*4. Ammeter in heater circuit, 0 to 3 amperes
*R*5. Variable resistor in heater circuit, 80 ohms, 2 amperes
*H*1. Heater coil immersed in bath liquid, 26 ohms
*N*1. Neon glow lamp across intermittent action leads, installed directly above thermometer-galvanometer scale
*M*5. Voltmeter across output from *R*8, 0 to 50 volts
*M*2. Milliammeter in output leads of *R*8, 0 to 50 milliamperes

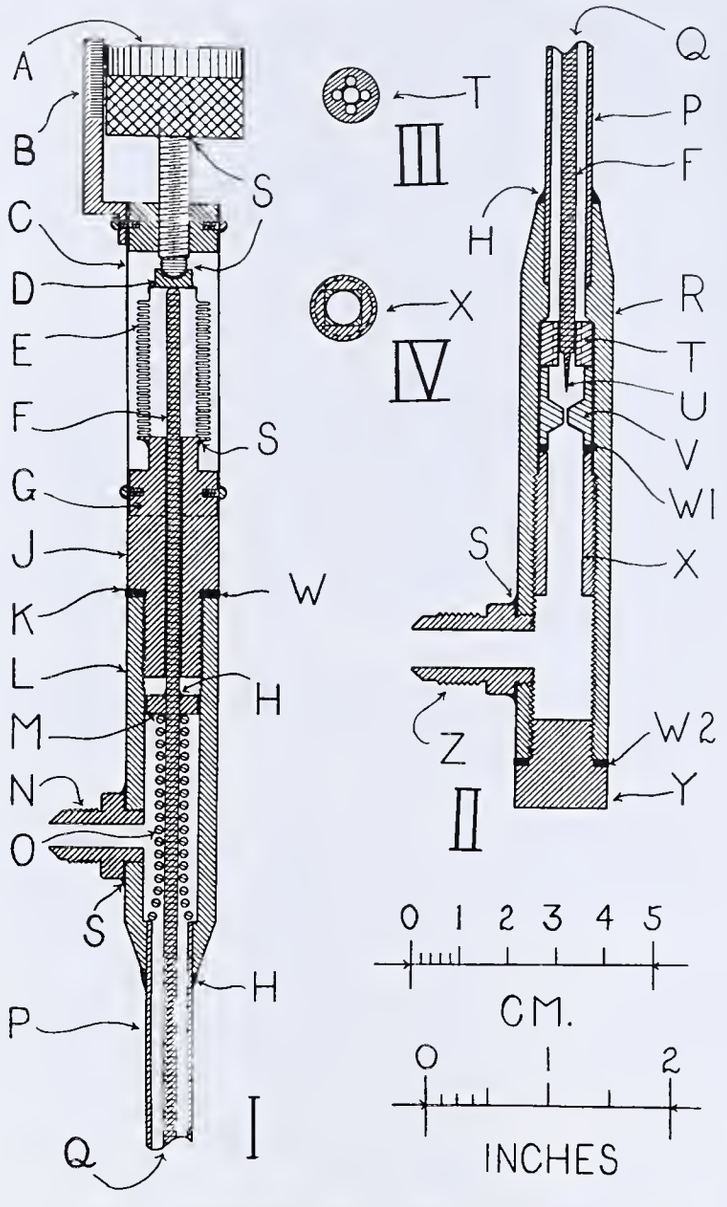

FIGURE 2. CROSS-SECTIONAL VIEW OF EXPANSION VALVE

Part I.

A. 50-division circular vernier engraved on top portion of knurled adjusting wheel
B. Turn counter, employed in connection with vernier
C. Tubing sheath for protection of bellows and for support of *A*, *B*
D. Thrust washer, soldered to *A*, free contact with *E*
E. Flexible circular bellows
F. Valve stem rod
G. Circular portion turned on end of hexagonal bar piece *J* to engage and support *C*
J. Hexagonal bar piece for support of upper structure, for providing a bearing for *F* and for engaging *L*
K. Joint made in assembly of all pieces
L. Hexagonal bar piece containing spring chamber and liquid refrigerant entry port
M. Thrust collar for *O*, soldered to *F*, slides freely in *L*
N. Standard refrigeration flare union for 0.63-cm. (0.25-inch) copper tubing, modified by cutting off portion of one end
O. Spring to keep *F* thrust against inside top part of *E*
P. Extension tube, connecting upper part of valve as shown in Part I with lower part as shown in II
Q. Joined to *Q* in Part II
W. Washer gasket

Part II.

F, *P*, *Q.* See Part I
R. Hexagonal bar piece forming body of expansion chamber
T. Centering bearing for *F*, drilled with holes to permit passage of liquid refrigerant
U. End of valve stem rod turned down to fit into orifice in *V*; regulation of vertical position permits varying amounts of liquid refrigerant to vaporize from chamber about *U* into chamber below *V*.
V. Orifice-containing piece
W1, *W2.* Washer gaskets
X. Thrust nut, to force gasket *W1* tightly against sliding-fit pieces *T* and *V*, making a seal against liquid refrigerant
Y. End nut formed of hexagonal bar; removal allows ready access to orifice chamber.
Z. Standard refrigeration flare union for 0.78-cm. (0.3125-inch) copper tubing, modified by cutting off a portion of one end; subsequently coupled onto bottom end of 0.9375-cm. (0.375-inch) copper tubing expansion coil through intermediary of a reducing union.

Part III.

T. End view of centering bearing, *T*, showing position of holes for liquid flow with respect to bearing hole

Part IV.

X. Bottom-end view of thrust nut, *X*, showing portion of center hole scraped out square; a special square-end wrench allows this nut to be inserted or removed from threading in *R*.

Common to all parts.

H. Brazed or hard-soldered joint
S. Soft-soldered joint

Material list.

W, *W1*, *W2.* Copper plate
O. Phosphor-bronze wire
F, *V.* Monel metal rod
M, *X.* Stainless steel rod
All others. Brass

Thread sizes.

1/4-inch × 40, on rod of *A*
7/16-inch × 20, S. A. E., on *N*
1/2-inch × 20, S. A. E., connection between *J* and *L*; on *X*, *Y*, and *Z*; inside bottom end of *R*

In the previous description of the cryostatic system, a commercial type of expansion valve was mentioned. This valve, although exhibiting good enough performance, had several undesirable features. The manual adjustment was much too critical; the size and shape of the instrument proper were such that it required a relatively large amount of space above the cryostat, this space being required for apparatus which was to be partially immersed in the bath liquid; the expansion of the refrigerating liquid took place above the surface of the bath liquid, the size and construction of the valve precluding direct immersion in the bath; an undesirable amount of frosting took place on the valve, in spite of heavy insulation; and the valve was of the diaphragm type, so constructed as to give a constant pressure in the evaporator coil. Since the pressure in the evaporator coil did not appreciably vary, owing to the action of the constant-pressure valve, *Q*, (Figure 2, *1*), this function was unnecessary, but the diaphragm was influenced by changes in barometric pressure and in the pressure of the liquid phase on the compressor high side due to changes in the room temperature; this latter change acted in contradistinction to that required for good modulation.

A new valve incorporates the following features: absence of a packed valve stem; small, compact size, with expansion occurring beneath the surface of the bath liquid; access to the orifice; fixed setting of the amount of refrigerant flow; countercurrent flow of warm liquid-phase refrigerant to the frosting process; resistance to corrosion; vernier adjustment of the setting; and standard refrigerant fittings employed wherever possible.

The packless valve-stem feature was considered advisable in order to eliminate the possibility of leaks, and was realized by employing a metallic bellows to transmit motion to the valve stem. The bellows specifications were as follows: 14.3-mm. outside diameter, 31-mm. free length with 25 active convolutions, fabricated from brass (80 per cent copper, 20 per cent zinc) of 0.114-mm. wall thickness. A motion of 7 mm. was obtainable on the compressive motion and the maximum internal pressure allowable was 14.3 atmospheres, a value somewhat in excess of that to be expected when in actual use. One end of the bellows was closed, and the open end was soldered onto a machined part that screwed into the valve assembly. (This bellows was a standard item fabricated by the Clifford Manufacturing Co., Boston, Mass., which soldered the bellows to the machined brass part and tested the subsequent joint.)

Hexagonal brass bar was employed for the main body parts of the valve, since it was then easy to use standard wrenches in assembling the constituent parts. Figure 2 shows a cross-sectional view of the valve, broken in two to avoid an unduly long drawing. The connection between the upper and the lower parts was about 25 cm., but any suitable length may be employed; for an exceptional length, it would appear necessary to have a centering bearing similar to *T*, in the connecting tube. In operation, the liquid refrigerant enters at *N* and passes through the interior of tube *P*, effectively preventing much frosting from taking place

n the exterior of the tube. The centering bearing, T, has four oles in it, in addition to the bearing hole for F, for the passage f the liquid refrigerant through the bearing piece. The actual xpansion from liquid to gas, or to mist, takes place through the rifice in V.

A commercial refrigeration halide leak tester has been found n invaluable adjunct for working with such an experimental ystem.

Acknowledgments

This work was done for Arthur B. Lamb in his laboratory nd the author wishes to thank him for permission to publish this description. W. W. Rice aided in testing the initial operation of the new expansion valve. The actual construction of the valve was effected by Alfred Gedies, the laboratory technician, to whom credit is due for his careful execution of the task and for certain suggestions, both factors contributing materially to the successful functioning of the instrument.

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An Improved Electrodialyzer

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ELECTRODIALYZERS on the market consist of three glass cells, clamped together in a specially designed tand, and in which two parchment or cellophane membranes eparate the middle cell from the outer cells. In addition o the fact that the middle cell has only a small capacity, he apparatus is somewhat difficult to handle, the dialyzing urface is small, and leaks may occur between the membranes nd the cells. It was desired, therefore, to design an electrodialyzer which would not only allow one to dialyze larger quantities of liquor, but would require little wash water for the electrodes—a distinct advantage when the material that dialyzes out is to be recovered by evaporation of the wash water.

An electrodialyzer which permits dialysis of larger amounts of liquids in a relatively short time and has been found to work satisfactorily is shown in Figure 1.

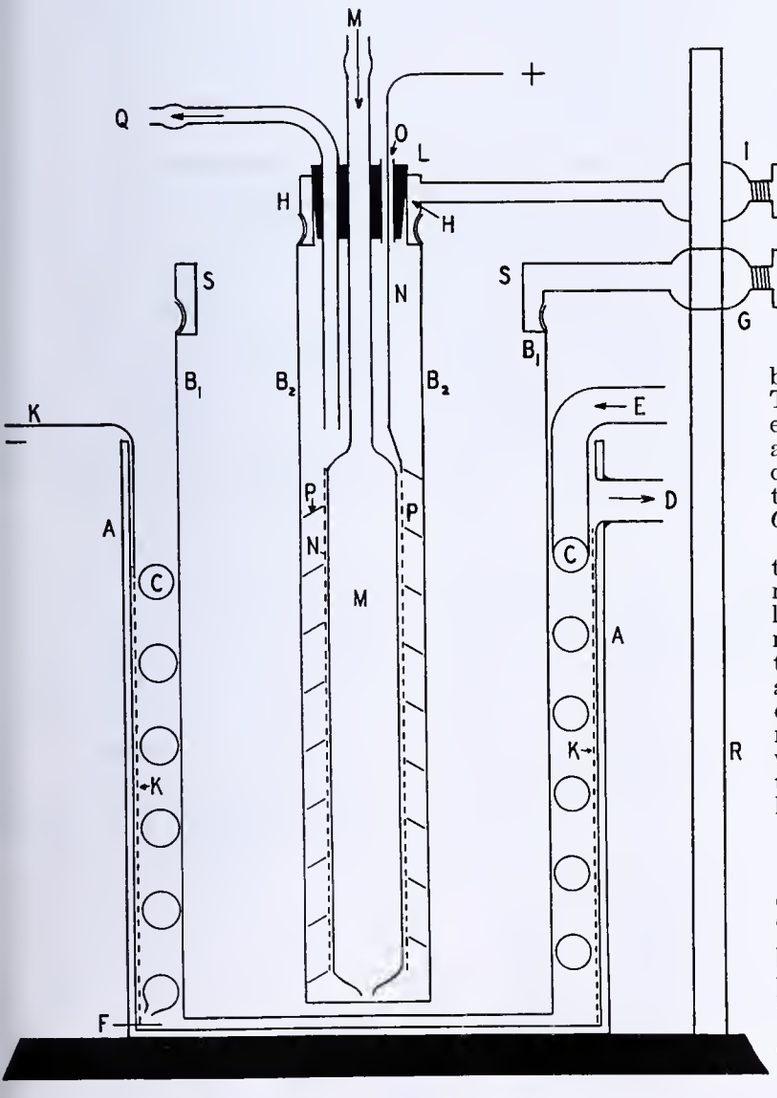

Figure 1. Diagram of Electrodialyzer

The three cells required in any electrodialysis apparatus are formed by the glass vessel, A, and the two dialyzing bags, B_1 and B_2. The outside cell, formed by A and B_1, has a platinum gauze electrode, K, which is laid against the glass wall of A and is used as a cathode. K is separated from B_1 by the glass tube spiral, C, which serves a double purpose. It prevents B_1, after it has been filled with the liquor to be dialyzed, from coming into contact with K and thus soon rendering the bag defective; further, the wash water for K is passed into the outside cell through this spiral. The water enters the spiral at E and the outside cell at F. Inasmuch as the spiral lies close to K on one side and B_1 on the other, the water is forced to pass through the outside cell along the spiral between K and B_1 and, in this way, causes a thorough washing of the electrode. The water leaves the outside cell at the overflow, D. The small distance between B_1 and K reduces the resistance of the wash water. The spiral fills a considerable amount of the space between the electrode and the bag, thus reducing the amount of wash water and increasing the concentration of the mineral content of the outside wash water. B_1 is suspended on a ring, S, which, in turn, is fastened to a ringstand, R, by means of the screw clamp, G.

The inner cell is formed by the dialyzing bag, B_2, which is tightly fastened to the ring, H, which, in turn, is screwed to the ringstand, F, at I. The glass tube, M, which is widened at its lower half, is inserted in B_2 and held in place by a three-hole rubber stopper, L. Around the outerside of the wider part of M, the electrode, N, is wound, the lead wire of which is led through and sealed in the capillary tube, O. In order to prevent any contact between B_2 and N, the latter is protected by the glass ribbon spiral, P, the ribbon of which forms an angle of about 70° with the spiral axis. The wash water enters the inner cell at the bottom of M, is forced by the glass spiral to rinse N, and leaves the cell through the glass tube at Q.

When hot water is passed through the outer and inner cells, the dialysis can be carried out at higher temperatures. The dialyzer can be used as an ordinary dialysis apparatus by taking out the inner cell at I. On account of its relatively large dialyzing surface, the dialysis proceeds rapidly and may be further accelerated by stirring the liquor in the middle cell.

Cotton cloth bags impregnated with viscose have proved most satisfactory as containers.

Photoelectric Photometer for Vitamin A Estimation

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VARIOUS methods have been devised for physically determining the amount of preformed vitamin A present in materials. One satisfactory type of measurement is based on the determination of the spectral absorption at 328 millimicrons, since Morton and Heilbron (*3*) found that the absorption of vitamin A–containing materials at that wave length was directly related to the potency of the material.

Numerous instruments have been devised for determining this absorption. One instrument, the Hilger Vitameter, employs visual estimation of the intensity of radiation transmitted by a solution of the vitamin A–containing material, the radiation being allowed to fall on a fluorescent screen. Absorption spectrograms have been used with densitometric determinations of the blackening of the photographic plate. These methods, while essentially correct, are approximate in the first case and tedious in the second and the latter requires relatively expensive equipment.

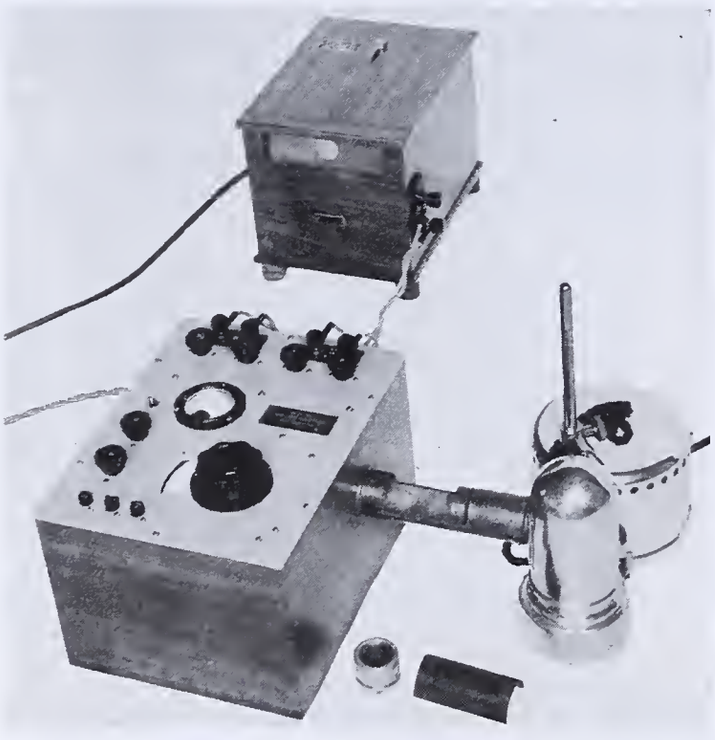

FIGURE 1. ASSEMBLED EQUIPMENT

Two simple instruments have been devised, one by Bills and Wallenmeyer (*1*) and the other by McFarlan, Reddie, and Merrill (*2*), which employ radiation in the vicinity of 328 millimicrons.

The instrument of Bills and Wallenmeyer utilizes an argon lamp as the source of radiation which with suitable filters isolates two bands, one slightly above and the other slightly below 328 millimicrons. This instrument requires calibration with materials of known potency, as readings are not strictly linear. The instrument designed by McFarlan, Reddie, and Merrill employs a sodium arc together with a red-purple Corex filter isolating radiation at 330 millimicrons. The radiation from this source is split into two halves, one of which passes through the test sample. In the case of this instrument it is necessary to balance the responses of the two photocells and amplify it. The adjustments determining the absorption of the material are based on the openings of diaphragms before the photocells.

The present paper describes the results of another investigation having for its object the construction of an economical instrument for this purpose, which would be portable, accurate, and simple in operation. Figure 1 shows a photograph of the equipment as it was finally assembled.

The sodium Lab-arc is shown, together with the controls and the tube in which the absorption cells are placed. The light transmitted through this cell chamber passes through a red-purple Corex A filter, No. 986.

Figure 2 is a schematic drawing of the circuit involved. A sodium photocell (G. E. No. FJ 405) sensitive to ultraviolet radiation is supplied with 90 volts and connected to the grid of the electrometer tube which has a grid leak of 100 megohms. This grid leak is not tied directly to ground but is brought through a slide wire to an appropriate potential relative to the filament of the electrometer tube. Variations in the grid potential will produce a variation in the current flowing through the electrometer tube which will correspondingly produce a deflection of the galvanometer.

The operation of the instrument consists in setting the filament rheostat, F, at an appropriate value (approximately 55-milliampere filament current). With the photocell dark and the slide wire set for a reading of zero transmission or infinite extinction coefficient, the bias rheostat, B, and the plate rheostats, P_1 and P_2, are adjusted for zero deflection of the galvanometer. Following this the illumination is permitted to fall on the photocell and the slide wire is set for 1.00 transmission factor or zero extinction coefficient. The resistors, S_1 and S_2, are then adjusted so that the galvanometer deflection is again zero. Interposition of the sample in the radiation beam will cause a deflection of the galvanometer, which is brought back to zero by means of the adjustment of the slide-wire potentiometer. The reading on the slide wire will then give either the transmission of the cell and ma-

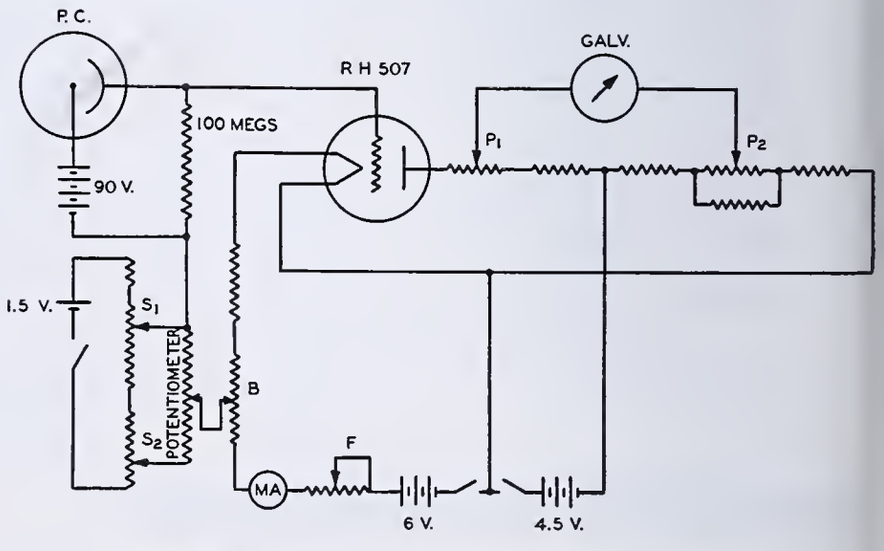

FIGURE 2. VITAMIN A PHOTOMETER CIRCUIT DIAGRAM

ABLE I. EXTINCTION COEFFICIENTS WITH VARIOUS DILUTIONS OF FISH LIVER OILS

(Cell of 1-mm. depth)

Ranges of potentiometer drum scale	1.5–3	4–6	6–8	8–10
Dilution number	1	2	3	4
Shark liver oil	19.0	18.7	18.7	18.7
Swordfish liver oil	20.8	21.0	20.4	21.2
Halibut liver oil	65.2	64.4	64.9	66.1
Tuna liver oil	66.7	66.6	66.4	66.7
Swordfish liver oil	94.0	98.0	98.5	98.2

ABLE II. COMPARATIVE *E* VALUES OF SOLUTION AT TWO CELL THICKNESSES

Sample	*E* Value 1-mm. cell	*E* Value 10-mm. cell
216	1.12	0.99
215	1.17	1.18
222	38.5	38.9
363	51.0	50.2
338	58.3	59.6
353	59.3	59.4
360	65.1	65.0

erial or their extinction coefficient. If a reading is first taken on he extinction coefficient scale, of the cell and solvent, subsequent readings taken of the cell, solvent, and active material ive the extinction coefficient of the cell and solution in question. ubtraction of the former from the latter gives the extinction oefficient of the material at the concentration employed.

Summarizing this procedure of operation, the principles nvolved are the maintenance of static conditions in the electrometer tube by holding the grid potential at a constant value. This is accomplished by the use of the slide-wire potentiometer. The accuracy of the readings depends on the calibration of the slide wire and this can be made with a precision of 0.25 per cent, far in excess of that required in this type of measurement. These operations, while somewhat lengthy of description, can be carried out extremely rapidly; in fact, only 5 minutes are required for three consecutive readings to provide an average value of the extinction coefficient of a solution.

The use of an absorption cell of either 1- or 10-mm. depth enables one to cover a wide range of vitamin A concentrations. A range of 150 to 1 can be covered in a single dilution with a precision of 2 per cent or better—that is, samples ranging from 1000 to 150,000 units of vitamin A per gram can be measured in a single dilution.

An instrument of this design has been in use at one of the authors' laboratories for approximately 9 months and has proved entirely satisfactory for these determinations. In order to provide evidence of this, data are given in the tables to show the linearity of the calibration, the reproducibility of measurements of a single dilution and of the same material over a period of time, and the correlation with other instruments and biological assays.

TABLE III. DUPLICATE DETERMINATIONS OF EXTINCTION COEFFICIENTS OVER A PERIOD OF WEEKS

Sample	Determination 1	Determination 2	Sample	Determination 1	Determination 2
1	0.39	0.37	12	4.70	4.88
2	0.71	0.68	13	14.46	14.75
3	0.91	0.92	14	28.4	29.6
4	1.30	1.29	15	37.4	37.3
5	1.43	1.36	16	56.3	57.0
6	1.47	1.43	17	58.2	56.7
7	1.55	1.60	18	63.6	62.9
8	1.62	1.64	19	66.8	66.5
9	2.00	1.97	20	67.1	66.8
10	2.04	2.09	21	86.8	86.2
11	2.86	2.78	22	137.2	140.4

Table I contains a series of typical readings on samples of fish liver oils originating from different species. Various dilutions of the materials were made and the extinction coefficients were determined from readings made at four widely separated positions on the potentiometer. The extinction coefficients obtained indicate that regardless of the type of fish liver oil or of the dilution, there is a linear relationship between potentiometer settings and concentration.

With respect to the reproducibility of the measurements, settings on the transmission scale may be duplicated to within ±2 parts of the scale which is divided into 1000 parts.

When determinations are made on a sample for cell depths of 1 and 10 mm., accurate linearity is found (Table II). Results are those obtained by reading the same solution at the two cell thicknesses.

TABLE IV. *E* VALUES OF OILS DETERMINED INDEPENDENTLY ON TWO INSTRUMENTS

Sample	Authors' Instrument	Monochromator Assembly
391	7.04	6.74
394	21.1	20.3
722	42.5	42.5
580	68.9	68.6
581	76.7	72.4
582	77.0	76.2
584	84.4	84.2
U. S. P. reference oil (whole)	1.56	1.45

TABLE V. COMPARATIVE POTENCY DETERMINATIONS

[Potencies (International units per gram) as determined by this instrument, by bioassay, and the average obtained by a group of laboratories (A. D. M. A. collaborators) employing physical methods of measurement]

Sample	Parker and Oser[a]	Average of Laboratories	Bioassay
1	3,000	3,074	3,000
2	1,500	1,713	1,500
3	61,800	61,595	70,000
4	31,200	31,401	35,000
5	298,000	285,027	300,000
6	143,000	143,740	150,000

[a] *E* value × 2120.

The reproducibility of results obtained at different times by an operator unacquainted with the duplication of the samples is shown in Table III, which lists the results obtained for samples varying in *E* value from 0.38 to 139.

Numerous interchecks have been carried out between this instrument and equipment consisting of a tungsten lamp as a light source, a monochromator set at a wave length of 328 millimicrons (10-millimicron slit width), and a photocell amplifier combination comparable to the one described above.

TABLE VI. VITAMIN A UNITAGE ESTIMATES FROM BIOASSAY DATA AND *E* VALUES

Sample	Assay Level *Units/g.*	Average Gains Assay *Grams*	Average Gains Reference *Grams*	Estimated Unitage[a]	$E^{1\%}_{1cm.}$	Calculated Unitage *E* × 2120
605	2,250	39.5	35.9	2,500	1.17	2,480
617	1,750	41.0	29.4	2,100	1.20	2,540
668	20,000	25.0	24.9	20,000	11.1	23,500
689	48,000	39.9	34.4	50,000	23.4	49,500
816	60,000	36.3	34.3	63,000	30.5	64,600
607	122,000	30.0	28.2	124,000	58.4	124,000
798	130,000	31.7	29.3	133,000	64.1	138,000
748	160,000	31.3	30.2	168,000	78.3	166,000
	168,000	32.2	32.5			
750	170,000	28.3	30.7	168,000	80.1	170,000
	180,000	25.7	30.7			
725	150,000	29.4	26.9	155,000	80.2	170,000
749	175,000	34.6	32.9	180,000	87.1	184,000
	185,000	30.4	33.1			
650	600,000	14.8	29.2	400,000[b]	337	715,000[b]

[a] Based on average dose-response curve from which unitage ratio corresponding to relative weight gains in assay and reference groups was obtained.
[b] This sample was a concentrate which may have undergone partial oxidation. It is not uncommon in such cases to observe lack of correlation between bioassay and physical measurement.

These checks (Table IV) show satisfactory agreement between the extinction coefficients determined by the two instruments.

At the meeting of the American Drug Manufacturers' Association in May, 1939, a report was presented of determinations of the extinction coefficients of six samples of oil, which were circulated among a number of collaborating laboratories. It had been hoped that the instrument described here would be available for use in the survey, but such was not the case. The authors therefore report the values subsequently obtained on this instrument for these samples. Table V lists the data obtained, together with the average values as determined physically by eight of these laboratories which were most consistent and the bioassay figures supplied to the collaborators.

Additional data bearing on the same point based on bioassays conducted at one of the authors' laboratories on a number of oils, together with the vitamin A potencies computed from measurements with this instrument, are given in Table VI.

Conclusion

The instrument here described makes possible rapid and accurate estimation of the amount of preformed vitamin A. It has been economically constructed, is readily portable, and does not require a high degree of technical skill in its operation or in the evaluation of the results. By the use of other light sources and filters, the instrument can be employed for making transmission measurements in other ranges of the ultraviolet; hence its scope is greater than for vitamin A assay alone.

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Presented before the Division of Biological Chemistry at the 99th Meeting of the American Chemical Society, Cincinnati, Ohio.

A Simple Vacuum Tube Relay

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THE successful operation of many recently developed laboratory and industrial instruments depends in a large part upon the operation of a sensitive relay control circuit. The control of heating elements or refrigeration equipment for the regulation of temperature is the outstanding example of the use of vacuum tube relays. Pressure-regulating manometers (*1*) and liquid-level controls (*4*) also require control circuits that operate with minute current flow or with a change in high resistance.

Several vacuum tube relay circuits have been recently offered (*2*, *3*, *5*, *6*) and for each is claimed one or more of the following advantages: simplicity of construction, low control current, wide range, ease of adjustment, continuous service, low cost, high degree of sensitivity, large plate current, power line operation, and alternating and direct current operation.

The relay described here has been designed to incorporate all these advantages as well as one or two others. The obvious advantage afforded by small size is realized in this circuit, which consists of one vacuum tube, four resistors, a condenser, and a commercial relay.

Schematic diagrams of the relay circuit connected for two

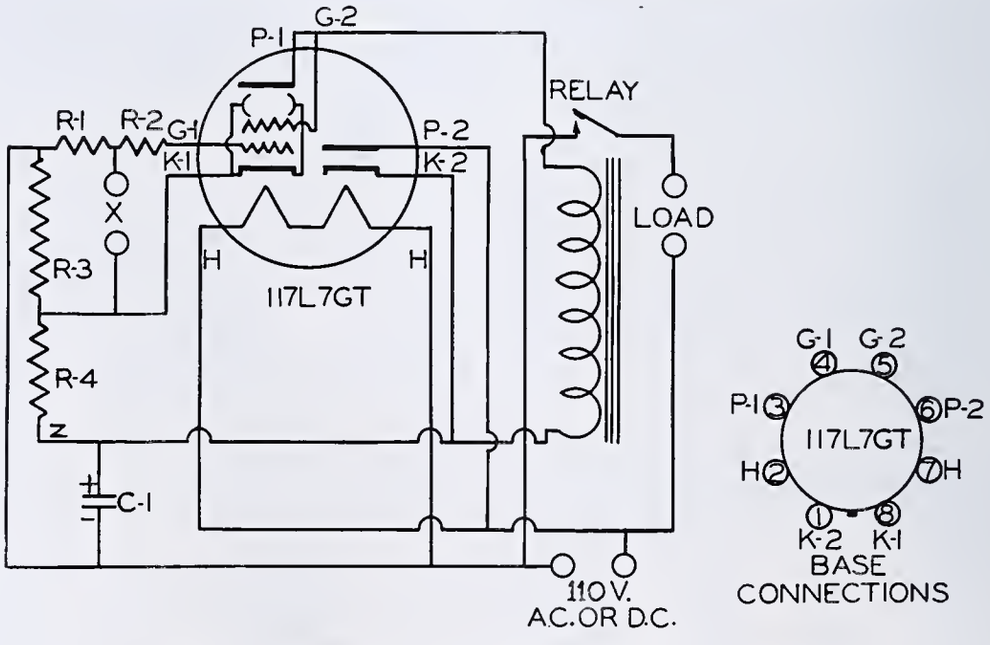

Figure 1. Schematic Diagram of Sensitive Relay Circuit

Make contacts to close load circuit
C-1. 8-mfd. electrolytic condenser, 250 working volts
R-1. 2-megohm, 0.5-watt resistor
R-2. 1-megohm, 0.5-watt resistor
R-3. 1000-ohm, 5-watt resistor
R-4. 15,000-ohm, 2-watt resistor
RELAY. Struthers-Dunn, Model ABTX1, 32-volt direct current coils (normally open)

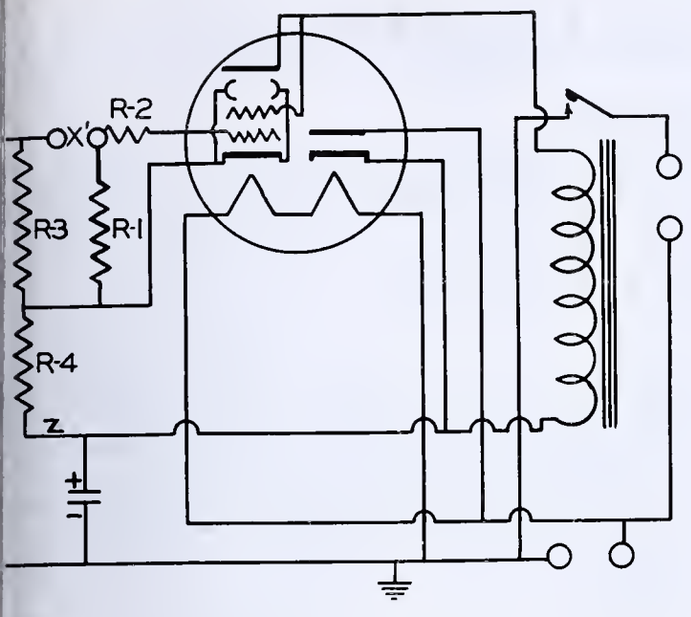

FIGURE 2. SCHEMATIC DIAGRAM OF SENSITIVE RELAY CIRCUIT
Make contacts to open load circuit

pes of operation are shown in Figures 1 and 2. Connection the circuit as shown in Figure 1 causes the relay magnet to come energized when the control contacts close or when ie resistance between the contacts becomes less than a cer- in value. The alternative connection in which the relay agnet opens when the contacts close is shown in Figure 2. oth types of circuits will operate with equal efficiency on ther alternating or direct current power supply lines.

The use of the rectifier-beam power amplifier type tube ype 117L7GT) serves the dual purpose of rectifying an ternating current supply and amplifying the minute con- ol current which flows through the contact points, *X*. ince rectified and partially filtered alternating current is pplied to the beam power amplifier section of the tube, aximum tube life and efficiency are assured. The direct rrent operation of the relay, obtained by this method, iminates the necessity of placing a condenser across the re- y coil to prevent chattering of the relay contacts. Instan- neous positive operation of the relay is thereby assured. he usual condenser may, however, be inserted across the re- y coil if delayed action is desired.

One of the outstanding features of this circuit is the use of vacuum tube, the filament of which operates directly from he 110-volt power line. The absence of the usual filament ransformer, lamp bulb, or other limiting resistor in the fila- ent circuit further simplifies construction and eliminates ne of the major sources of trouble in many relay circuits.

The very high plate current supplied by the beam power mplifier (45 to 50 milliamperes) eliminates the necessity for he use of a sensitive relay. The inexpensive and rugged ommercial type of relay suggested for this circuit assures ositive operation at all times for loads up to 1000 watts. lthough high plate current flow is obtained, the current flow cross the control circuit contacts, *X*, is limited to a maximum f about 5 microamperes. If an external resistance is in- erted in the control circuit, this control current may be de- reased, although the necessity for this precaution seems un- kely.

Since fixed resistors are used in the construction of the re- ay circuit, no electrical adjustments need be made. Me- hanical variation of the spring tension on the relay contacts nay be desired for different types of operation. Should a vider range of adjustment be desired for operation with a different type of relay, resistor *R*-3 may be replaced by a 1000-ohm wire-wound potentiometer.

Either relay circuit may be adapted to uses which require the control circuit to operate the relay with a change in control resistance. The relay of the circuit shown in Figure 1, for example, will remain energized so long as the resistance in the control circuit (across *X*) does not exceed approximately 500,000 ohms. If the control circuit resistance exceeds this value the relay magnet will open the load circuit. Variation of the critical resistance may be obtained by adjustment of the relay contact spring tension.

The simple and compact construction which may be realized with this type of relay is shown in Figure 3. The total cost of parts necessary for this model is less than $7. Standard radio replacement parts are used throughout.

The circuit shown in Figure 1 may be adapted for operation as a photoelectric cell relay. The cathode of a high-

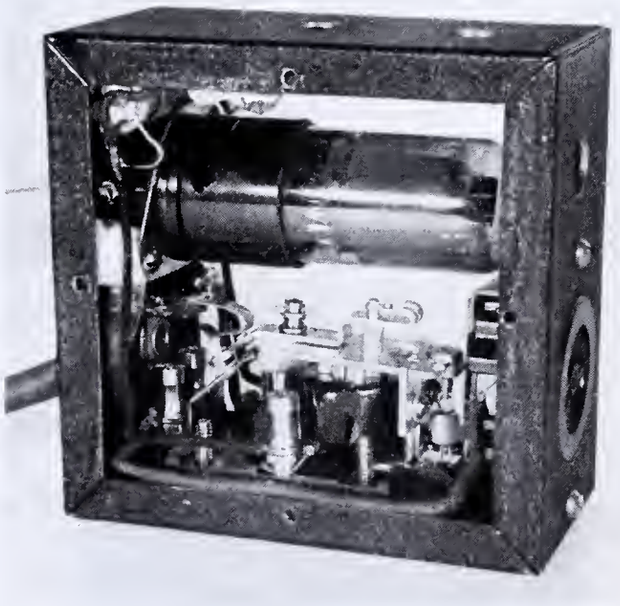

FIGURE 3. COMPLETED RELAY UNIT IN 4 × 4 × 2 INCH CASE

resistance photocell is connected to *G*-1 and the anode is connected at point *Z* in Figure 1. Light striking the photoelectric cell will cause the relay to become energized, providing that contact points *X* are open.

Since the circuits described are not isolated from the power supply line, care must be exercised in handling the control circuit contacts. If one side of the regulator is to be grounded, the grounded side of the power line and regulator must be connected as shown in Figure 2.

About twenty of these relays have been constructed and installed for various types of operation. A number of these units have been subjected to continuous 24-hour operation for 10 months with no apparent change in sensitivity. Not a single failure of any tube or part has been reported during this time.

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Device for Continuous Liquid-Liquid Extraction

Adaptation for the Determination of Morphine

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CONTINUOUS liquid-liquid extraction devices for use with immiscible solvents lighter than water have been described in numerous publications.

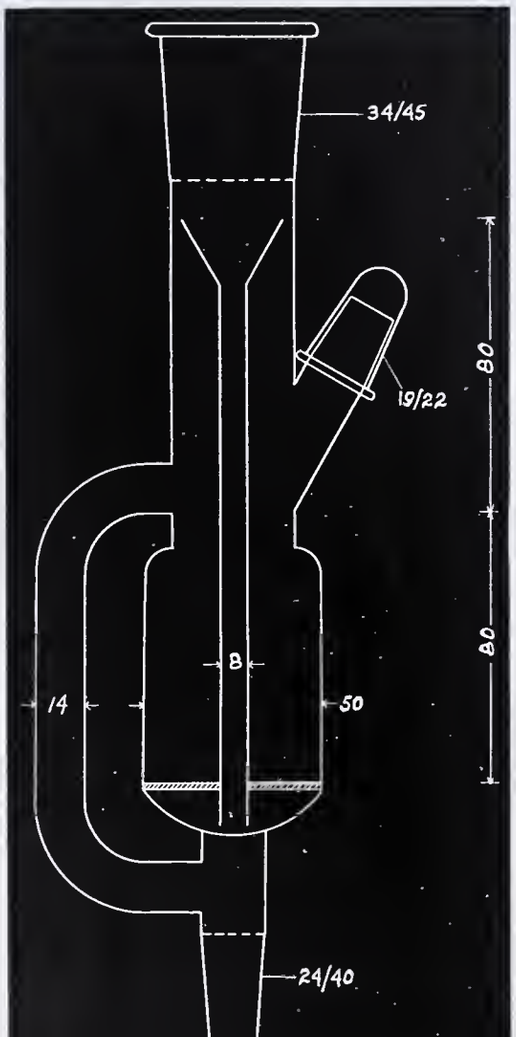
The most useful of these for the quantitative extraction of small samples are patterned after the general design of Palkin, Murray, and Watkins (*1*), in which solvent is conveyed from a condenser to the bottom of the solution being extracted, from whence it rises through the solution and returns to the boiling flask. Sintered-glass plates, spirals, small orifices, and the like are used for distributing the solvent throughout the solution. These distributing devices are generally separate from the body of the extractor and, being fragile, are consequently subject to breakage; but more serious disadvantages are that solvent is distributed from a relatively small area and a space exists at the bottom of the extractor where solvent and solution do not come into contact.

Under these conditions, much time is required for complete extraction and, in this laboratory, it has been found necessary to disconnect the apparatus from time to time to stir the solution being extracted, in order to obtain quantitative results. It is necessary also to disconnect the apparatus for the purpose of withdrawing samples of solvent in order to determine when the extraction is complete. These operations are annoying and time-consuming, especially since one must wait until the solvent stops boiling before removing the condenser.

The device described herein has been designed to obviate these difficulties. It has been found both quick and convenient for quantitative extraction of alkaloids, and is equally well suited for other substances.

The sintered-glass solvent-distributing plate is permanently sealed in the extraction tube where it covers the entire cross section and is protected from breakage. Solvent is distributed throughout the entire volume of the solution being extracted rather than in a narrow stream of droplets. There is no space under the distributing plate where solvent and solution do not come into contact.

The apparatus is filled and emptied through a side arm closed by a ground joint which also provides an opening through which to pipet occasional samples of solvent to test for completeness of extraction. The opening is sufficiently small to permit removing samples without interrupting the boiling. A drawn-out glass tube provided with a rubber bulb is convenient for this purpose.



Apparatus

The diagram is self-explanatory. In operation a boiling flask for the solvent is attached by the ground joint at the bottom and a good condenser is attached in similar fashion at the top. The solvent vapors distilling from the boiling flask are condensed, passed down the inner funnel, and distributed throughout the aqueous solution by means of the sintered-glass plate. Solvent returns from the upper layer in the extractor to the boiling flask through the same tube which carries the vapors to the condenser. The device is charged and emptied through the side opening. The dimensions can be modified to almost any desired extent.

For most satisfactory operation it is important that the sintered plate be as coarse as possible, that the funnel tube extend as nearly as possible to the bottom of the extraction tube, and that the space between the plate and the bottom be small.

Determination of Morphine

The device has been found particularly useful for the determination of morphine, where it presents a number of advantages over the generally used method of extracting the alkaloid from an ammoniacal solution by a mixture of chloroform and isopropanol. This operation is not readily adaptable to a continuous process where the solvent is recycled by boiling and condensation, because chloroform hydrolyzes under these conditions and the resulting acids unite with the extracted morphine, thus vitiating the results. The hand operation, employing separatory funnels, requires much solvent, is extremely tedious, and permits extraction of only small samples.

A mixture of benzene and isopropanol (2 volumes to 1 volume) serves admirably as extracting solvent in the device

lescribed. Only small volumes are required and relatively arge samples (about 0.20 gram of alkaloid) are readily ex-racted.

Place sufficient solvent to cover the sintered plate in the device, ntroducing it through the inner funnel. Accurately weigh a sam-ple equivalent to approximately 0.20 gram of morphine alkaloid nd dissolve it in approximately 50 cc. of dilute hydrochloric or ulfuric acid. Quantitatively transfer the solution to the ex-ractor through the side opening, using approximately 25 cc. of vash water.

Place 50 to 75 cc. of solvent and a few Carborundum chips n the boiling flask and connect flask to the extractor. Drop ufficient ammonia to render the solution faintly alkaline through he inner funnel and connect the condenser. Distill the solvent apidly through the solution until a sample of solvent removed (through the side tube by means of a pipet) from the upper layer n the extractor is found by a suitable test (Marquis reagent) to e free of morphine. The presence of sufficient ammonia in the olution may be confirmed at the same time by holding a piece f wet litmus paper in the vapor escaping from the side tube. When extraction is complete (0.5 to 1 hour) disconnect the oiling flask, evaporate the solvent, and titrate the alkaloid in the sual way.

Under the conditions described there is a volume increase of 20 per cent in the aqueous layer due to solubility of isopropanol. The addition of ammonia in the prescribed manner renders the solution alkaline at the same time that solvent is available to extract the liberated alkaloid. This is of consequence in morphine extraction and presumably also in other cases.

The simple analytical procedure outlined is, of course, useful only where interfering substances are absent. When this is not the case, the extractor becomes even more useful. Morphine can be separated from many other alkaloids, for example, by dissolving the mixture in dilute acid, rendering the solution distinctly alkaline with sodium hydroxide, and extracting with benzene to exhaustion. The solution is acidified, then made alkaline with ammonia, and the morphine removed in fresh mixed solvent (benzene-isopropanol). A method for opium assay based on this procedure is now under consideration in this laboratory.

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Perforated Plate Columns for Analytical Batch Distillations

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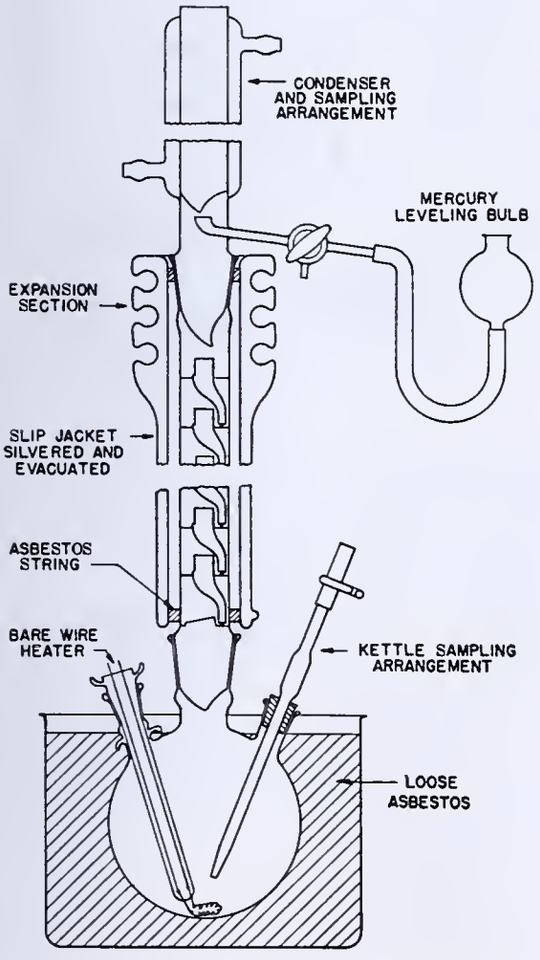

Figure 1. Perforated Plate Column

ACCURATE analytical batch distillations have become increasingly important during the past few years. Of the many different columns tested in these laboratories for this purpose, the perforated plate column described in this paper has been found to be of especial value for the analysis of hydrocarbon mixtures. It combines a low operating holdup per theoretical plate and a negligible static, or nondrainable holdup, with a high capacity or reflux rate. It has a low H. E. T. P., is easy to operate, and since it is constructed entirely of glass, may be used for corrosive liquids.

Description of Column

The column (Figures 1 and 2) consists of a series of perforated glass plates sealed into a tube. Each plate is equipped with a baffle to direct the flow of liquid, a weir to maintain a liquid level on the plate, and a drain pipe. The first plate in a series serves as a small reservoir which is necessary in order to maintain a liquid seal for the drain pipe from the first regular plate.

Construction

The plates are constructed in the following manner: A tube which later will form the baffle is placed in a lathe and rapidly rotated. The protruding end of the tube is heated in an oxygen flame and flared to form the disklike portion of the plate. The perforations are then drilled with a red-hot tungsten wire. This operation, which is ordinarily somewhat time-consuming when done by hand, is now entirely performed by an automatic drilling device designed in these laboratories for the purpose. The hot tungsten wire forms a small burr on the plate which is removed with an ordinary file. After the drilling operation the plates are examined and only those having perforations of uniform diameter and spacing are selected for use. The selected plates are ground to size; if they have been correctly flared, the grinding is a minor operation.

The drain pipes are sealed in place and shaped as shown in Figure 3, and a bead 0.5 mm. high is made on the lower end of each to regulate the distance between the drain and the plate below. The baffle tube is cut off at the desired height above the plate, and two longitudinal cuts are made with small high-speed

FIGURE 2. SECTION OF PERFORATED PLATE COLUMN SHOWING DETAILS OF PLATE CONSTRUCTION

emery wheels correctly spaced on a shaft. The intervening segment of the baffle is then cut off 1 mm. above the plate to form the weir. The plates are made up in large batches and carefully annealed between operations, when necessary, to remove strains. The finished plates are examined for imperfections.

The selected plates are then carefully sealed into a tube, so that each plate is level and the bead on the bottom of each drain pipe rests firmly on and in the center of the unperforated section of the plate below. The bottom of the pipe is placed sufficiently close to the plate below to ensure a liquid seal under normal operating conditions. The drain pipe is tapered from top to bottom to prevent vapor bubbles from being trapped by the overflowing liquid.

The column may be insulated in a number of ways. A slip jacket of the type shown in Figure 1 has been developed and is generally preferred in these laboratories. The jacket has a narrow window running its entire length through which the operation of the column may be observed.

Testing the Column

PLATE EFFICIENCY. Plate efficiencies were determined at several reflux rates on four columns, Nos. 1, 2, 3, and 5.

Columns 1, 2, and 3 are 25 mm. in diameter. Each plate in column 1 has 42 perforations, 0.85 mm. in diameter; those of column 2 have 44 perforations, 1.1 mm. in diameter; and those of column 3 have 44 perforations, 1.35 mm. in diameter. The plate spacing is 25 mm. in columns 1, 2, and 3. Column 5 is 32 mm. in diameter. Its plates have 81 perforations, 0.85 mm. in diameter, arranged in three concentric circles. The plates are spaced 30 mm. apart in the column to give greater flexibility of operation. A carbon tetrachloride–benzene mixture prepared from redistilled Baker's C. P. chemicals was used in these tests.

In the plate-efficiency tests, the compositions of still-head and kettle samples were determined by the method of refractive index.

Still-head samples were withdrawn by means of the special still head shown in Figure 1. The mercury leveling bulb was lowered, thereby drawing some liquid through the three-way stopcock, the stopcock was then turned, and a few drops of sample were forced out by raising the mercury level. The remainder of the liquid in the line was then forced back into the column by displacing it with mercury. Kettle samples for the tests on columns 1, 2, and 3 were withdrawn by means of the pipet shown in Figure 1. However, in testing column 5, samples were taken from the bottom plate of the column instead of the kettle by means of the reflux rate and holdup measuring device shown in Figure 5. This apparatus is similar to that described by Bragg (*1*).

The plate efficiencies were determined by the method of McCabe and Thiele. In order to determine when equilibrum was obtained, still-head samples were analyzed at intervals. When successive samples showed constant composition, still-head and kettle samples were taken simultaneously and analyzed. The number of theoretical plates was determined from a diagram constructed from the vapor-liquid equilibrium data of Rosanoff and Easley (*5*). The kettle was taken as equal to one theoretical plate; therefore, the numbers of plates in columns 1, 2, and 3 are given as one less than the total number found. The method of sampling used in testing column 5 gave directly the number of plates in the column.

REFLUX RATE. The reflux rates at which the plate-efficiency tests were made were determined on columns 1, 2, and 3 at the end of each test by simply replacing the sampling still head with the total take-off head shown in Figure 4 and measuring the liquid collected in a given time. The reflux rate and holdup measuring device shown in Figure 5 was used in all tests on column 5. Therefore, the reflux rates at which plate efficiencies were determined on column 5 were measured by simply closing the stopcock in the reflux return line. In this case the volume was determined at the boiling point of the liquid. The results of these tests are given in Table I.

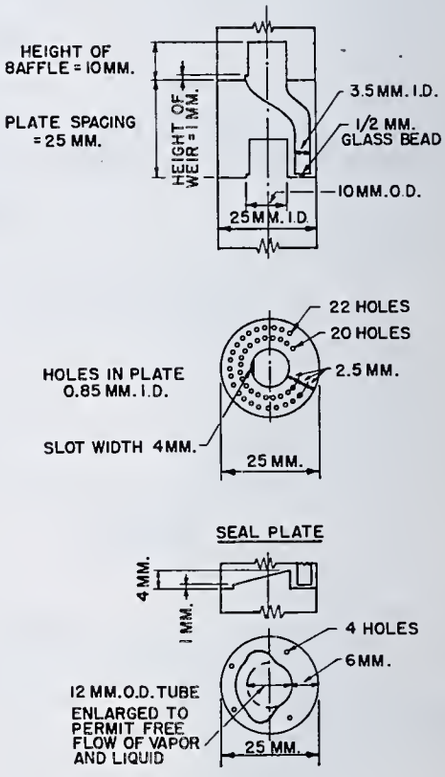

FIGURE 3. PLATE CONSTRUCTION

OPERATING HOLDUP. Operating holdup was determined at several reflux rates on columns 4 and 5. Column 4 is identical with column 1 except that it has 37 actual plates instead of 10. The reflux rate and holdup apparatus (see Figure 5) was used in these tests; Baker's C. P. benzene was used as the test liquid. The reflux rates were determined in the manner described above. The operating holdup was determined by closing the stopcock in the reflux return line and shutting off the heater simultaneously, thus allowing the liquid in the column to drain into the graduated portion of the apparatus. This method of determining the holdup was made possible by the negligible heat capacity of the bare wire immersion type heater. (This type of heater has been found very satisfactory for distilling materials that are not affected by platinum or chromel resistance wires. It is sensitive to control, constant in operation, and presents essentially no fire hazard. The volumes were measured at a temperature very near the boiling point of the liquid. The results are given in Table II

TABLE I. EFFICIENCY TESTS

Reflux Rate		CCl_4 Kettle	CCl_4 Still head	No. of Theoretical Plates in Rectifying Section (Total Plates − 1)	Plate Efficiency	H. E. T. P.
Ml./min.	*Moles/min.*	*Mole %*	*Mole %*		*%*	*Inches*
Column 1, 10 25-mm. plates, 42 0.85-mm. perforations per plate						
6	0.065	15.4	44.0	10.0	100	0.98
13	0.141	16.7	42.5	8.9	89	1.10
18	0.195	19.0	45.2	8.8	88	1.12
24	0.260	16.7	42.0	8.6	86	1.14
Column 2, 10 25-mm. plates, 44 1.1-mm. perforations per plate						
15	0.162	18.0	43.0	8.4	84	1.17
26	0.282	18.0	41.0	7.7	77	1.28
28	0.304	18.0	40.5	7.5	75	1.31
Column 3, 10 25-mm. plates, 44 1.35-mm. perforations per plate						
12.5	0.136	14.8	37.5	7.8	78	1.26
23.5	0.255	14.8	34.5	6.7	67	1.47
Column 5, 32-mm. plates[a], 81 0.85-mm. perforations per plate						
		Below plates	Still head	No. of Theoretical Plates in Column		
18.3	0.190	18.6	57.4	15.3	102	1.16
36.8	0.381	21.2	55.1	13.0	87	1.36
39.0	0.404	17.0	50.3	12.7	85	1.39
52.0	0.540	17.0	48.0	11.7	78	1.51
55.5	0.575	17.0	47.4	11.5	77	1.53
70.5	0.730	19.2	46.8	10.3	69	1.71

[a] Plates spaced 3.0 cm. apart in column.

TABLE II. TESTS OF HOLDUP AND REFLUX RATES

Reflux Rate		Holdup in Column
Ml./Min.	*Moles/min.*	*Ml.*
Column 4, 37 25-mm. plates, 42 0.85-mm. perforations per plate		
9	0.094	20
14	0.146	26
22	0.229	53
28	0.292	58
40	0.417	67
Column 5, 15 32-mm. plates, 81 0.85-mm. perforations per plate		
17	0.177	14
28	0.292	38
52	0.542	40
75	0.782	45

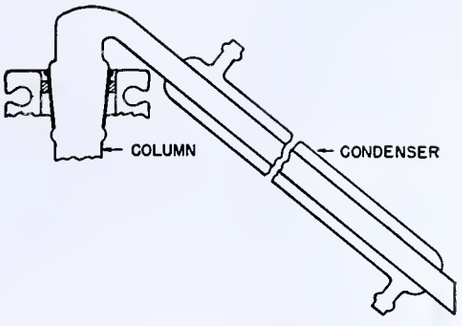

FIGURE 4. TOTAL TAKE-OFF STILL HEAD

instead of the holdups were determined by extrapolation.

The reflux rates in moles per minute were calculated from the molar volumes of the liquids and the reflux rates in milliliters per minute. The molar volume of benzene at 80° C., the approximate temperature at which the volumes were measured, was calculated from the molar volume at 20° C. by means of the data given by Perry (*4*) on the coefficient of thermal expansion. For mixtures of carbon tetrachloride and benzene and of ethylene dichloride and benzene, the molar volumes were determined from graphs of molar volume *vs.* molal composition. In calculating the molar volumes at the approximate boiling points of the mixtures it was assumed

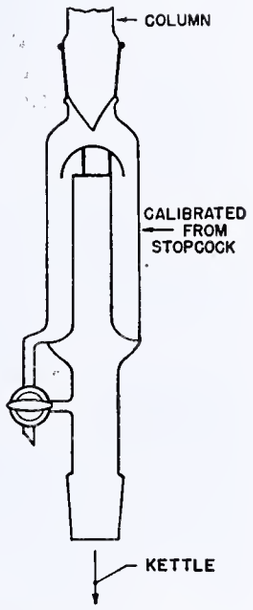

FIGURE 5. REFLUX RATE AND HOLDUP TEST APPARATUS

The performance data in Table III have been calculated from the data given in Tables I and II and Bragg's data (*1*) on the Stedman type packing which has been included for purposes of comparison. Similar data on columns of the helix-packed type (*3*, *7*, *8*) could not be calculated from the information given in the literature. Data on the Bruun column (*2*) were also omitted, since the operating holdup was not given for a specified reflux rate.

PRESSURE DROP. The pressure drop per theoretical plate was not measured; however, it is to be expected that the pressure drop per theoretical plate in a plate column will be higher than that in a packed column. The maximum pressure drop may be calculated approximately from the density of the refluxing mixture, the plate spacing, and the plate efficiency. Thus, for a mixture whose density is 0.8 at its boiling point, and which has a plate spacing of 25 mm. and a plate efficiency of 80 per cent, the maximum pressure drop per theoretical plate would be approximately 1.8 mm. of mercury.

Methods of Calculating Data

The plate efficiencies given for column 4 in Table III were determined by extrapolating and interpolating those found for column 1 to the molal reflux rates at which operating holdups were determined. The operating holdups given for column 5 in Table III were determined by interpolating those given in Table II. In the case of the highest and lowest reflux rates given for column 5 in Table III, the plate efficiencies

TABLE III. PERFORMANCE OF PERFORATED PLATE AND STEDMAN TYPE PACKED COLUMNS

Reflux Rate		No. of Theoretical Plates in Column	Plate Efficiency	H. E. T. P.	Holdup		
					Total	Per theoretical plate	Per theoretical plate per 100 ml. of reflux per min.
Ml./min.	*Mole/min.*		*%*	*Inches*	*Ml.*	*Ml.*	*Ml.*
Column 4, 37 25-mm. plates, 42 0.85-mm. perforations per plate							
9	0.094	35.1	95	1.04	20	0.57	6.4
14	0.146	32.9	89	1.10	26	0.79	5.7
22	0.229	32.2	87	1.13	53	1.65	7.5
28	0.292	31.8	86	1.14	58	1.83	6.5
40[a]	0.417	30.0	81	1.21	67	2.23	5.6
							Av. 6.3
25-mm. Stedman column, 24 inches of packing 112							
3.33	0.0394	43.8	...	0.55	13	0.30	8.5
6.67	0.0790	34.8	...	0.69	22.4	0.64	9.3
8.33	0.0985	31.1	...	0.77	24.0	0.77	8.8
11.65	0.138	29.5	...	0.81	27.0	0.92	7.6
15.0	0.177	26.3	...	0.91	33.0	1.25	8.0
16.7	0.197	24.6	...	0.98	34.0	1.38	8.0
18.0[a]	0.213	24.2	...	0.99	35.8	1.48	7.9
							Av. 8.3
Column 5, 15 32-mm. plates[b], 81 0.85-mm. perforations per plate							
17.0	0.177	15.5	103	1.14	14	0.90	5.3
18.3	0.190	15.3	102	1.16	17	1.11	6.2
36.8	0.381	13.0	87	1.36	39	3.00	8.1
52.0	0.542	11.7	78	1.51	40	3.42	6.6
70.5	0.730	10.3	69	1.71	44	4.37	6.2
75.0[a]	0.782	10.0	67	1.76	45	4.50	6.0
							Av. 6.4

[a] Maximum reflux rate.
[b] Plates spaced 30 mm. apart in column.

that the coefficient of thermal expansion of the mixtures was the same as that for benzene.

Table IV. Comparison of Perforated Plate with Stedman Type Packed Columns

	25-Mm. Perforated Plate Column	25-Mm. Stedman Column
Holdup ml. per theoretical plate per 100 ml. of reflux per minute (average)	6.3	8.3
Reflux rate, mole per min., maximum	(0.094)[a]–0.417	(0.039)[a]–0.213
H. E. T. P., inches	1.04–1.21	0.55–0.99

[a] This figure represents reflux rate at which the low H. E. T. P. was determined and not minimum reflux rate of column.

In calculating the holdup per theoretical plate per 100 ml. of reflux per minute, the reflux rate was taken as an average of that at the top and bottom of the column by assuming that the variation in rate was due to the change in molar volume with composition; changes in rates due to variation in heats of vaporization were neglected. The average reflux rates in milliliters per minute calculated in this manner for mixtures of ethylene dichloride and benzene were 4 per cent higher than those given by Bragg. It has been learned through private communication that the volumes of holdup reported by Bragg were measured near the boiling point of the liquid, and consequently no correction for expansion has been made.

Discussion of Data

Size of Perforations. The data in Table I show that at comparable reflux rates the plate efficiencies decrease with an increase in the size of the perforations, and that with perforations of a given size the plate efficiencies vary inversely as the reflux rate. An examination of the data on plate efficiencies to be expected in the 25-mm. columns at a reflux rate of 0.25 mole per minute gives the following results:

0.65-mm. perforations, 90% by extrapolation
0.75-mm. perforations, 88% by extrapolation
0.85-mm. perforations, 86%
1.1-mm. perforations, 79%
1.35-mm. perforations, 68%

The 0.65-mm. perforations could be expected to increase the plate efficiency by 4 per cent over that of the recommended 0.85-mm. perforations, but on the other hand, the pressure required to force vapor through smaller perforations against the surface tension of the liquid is higher. Therefore it is necessary to increase the spacing of the plates and consequently to increase the H. E. T. P. The use of perforations larger than 0.85 mm., however, was found to decrease the flexibility in reflux rate.

The plate spacing of 25 mm. recommended for column 1 represents actually a compromise between flexibility and H. E. T. P. In the case of column 5, which was built for continuous as well as batch distillations, a plate spacing of 30 mm. was used because flexibility was considered to be of greater importance than a low H. E. T. P.

Holdup and Comparison of Perforated Plate with Other Types of Columns. It may be seen from Tables I and III that the operating holdup per theoretical plate varies with the reflux rate. However, if the holdup per theoretical plate is divided by the reflux rate in milliliters of liquid per minute, a practically constant value is obtained. Now if the capacity or maximum reflux rate of the columns is expressed in moles per minute, a useful basis for the comparison of batch distilling columns of different design is established. For example, a comparison of the perforated plate column with the Stedman column by this method gives the results of Table IV.

Of the three factors given above, the holdup per theoretical plate at a fixed reflux rate is the most important in choosing a column for analytical batch distillations, since it determines the minimum volume of liquid which must occur as intermediate fractions between two essentially pure components. Thus the minimum intermediate fraction which must exist in the Stedman column would be approximately 32 per cent larger than that in the perforated plate column if both were operated at the same reflux rate. It follows that, if intermediate fractions of equal size are desired in both cases, the perforated plate column could be operated at a higher reflux rate, thus decreasing the time required to effect the separation. The actual volume of the intermediate cut could be decreased in both the perforated plate and the Stedman columns by decreasing the reflux rates. A detailed discussion of the effect of holdup and also of the effect of reflux ratio in batch distillations is given by Rose (*6*).

The figures obtained for reflux rate and H. E. T. P. show that the 25-mm. perforated plate column has a somewhat greater H. E. T. P. than the 25-mm. Stedman column and that the perforated plate column has a higher maximum reflux rate or capacity. Comparison with the helix-packed type of column (*8*) shows that the perforated plate column has a lower average holdup per theoretical plate per 100 ml. of reflux per minute. Comparison with the Bruun column (*2*) shows that the perforated plate column has a higher plate efficiency at high reflux rates. Since the liquid drains readily and practically completely from the perforated plate column, it is superior in this respect to the Bruun column.

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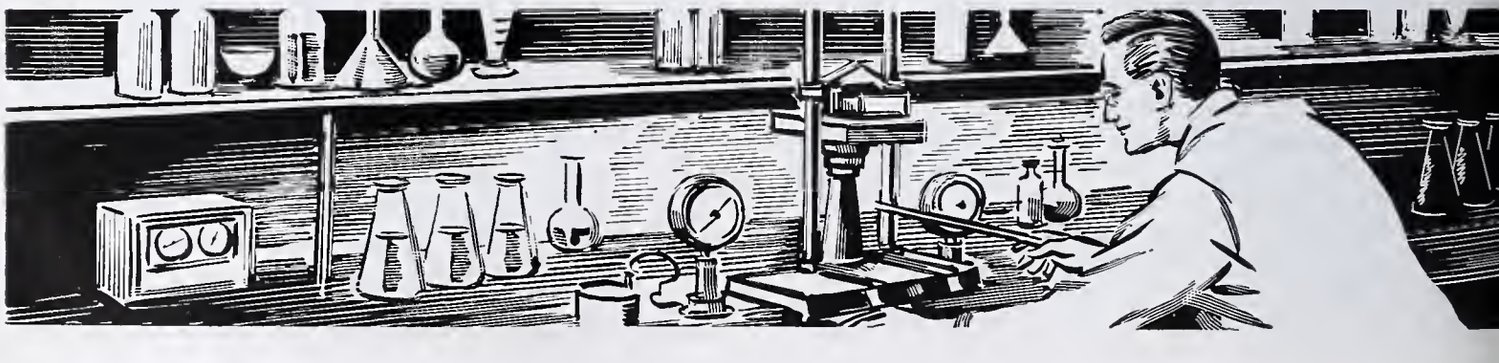

Microanalysis of Gaseous Hydrocarbons

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IN THE study of gaseous reactions the gaseous products are often obtained in such small quantities that ordinary methods of gas analysis may not readily apply. Furthermore, these methods do not render it possible to differentiate between a homogeneous gaseous hydrocarbon and a mixture of hydrocarbons.

A procedure has therefore been developed applying Pregl's microanalytical method for the determination of carbon and hydrogen to the analysis of gaseous hydrocarbons obtained in such small quantities. By introducing about 2 cc. of the gas into the combustion tube by means of a buret inserted into the Pregl train and slightly modifying the well-known procedure, extremely good results are obtained. As in the ordinary microdetermination (*1*, *2*) of carbon and hydrogen in organic substances, the water and carbon dioxide are weighed. From these the weights of carbon and of hydrogen are calculated and the sum of the two is obviously equal to the weight of the sample taken. This weight makes it possible not only to determine the percentage composition but to calculate the molecular weight of the hydrocarbon from the relation of the weight of the sample to the volume of gas taken.

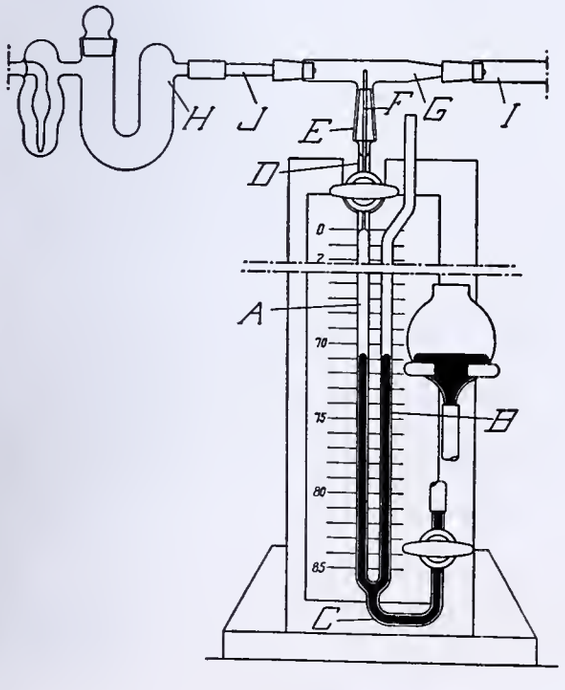

FIGURE 1

Apparatus

The gas buret designed for this purpose is illustrated in Figure 1. It consists of a glass tube, *A* (3-mm. bore), approximately 1 meter in length, branched at its lower end into a parallel compensating tube, *B*, and a short tube, *C*, which is closed with a stopcock and connected to a leveling bulb filled with mercury. The upper end of *A* is sealed to a capillary, *D* (1-mm. bore), carrying a stopcock and terminating in a ground tip, *E*, inside of which there extends a fine capillary tube, *F* (0.5-mm. bore), which is sealed to *D*. A piece of glass tubing, *G*, of the same diameter as the Pregl combustion tube, *I*, is drawn at one end to a tip of the size of the thermometer capillary, *J*, prescribed by Pregl (*2*, p. 26) to join the bubble counter, *H*, to *I*. *G* is connected to *E* by means of a ground-glass joint and can be secured in place with springs held by small glass projections on the two pieces of the joint. *F* should be sufficiently long to reach the center of *G* as shown. The buret is mounted on a supporting board on which a strip of graph paper has been pasted and is inserted in the combustion train between *J* and *I*. The buret is calibrated and the relation of centimeter length to volume is plotted on a graph which can be used to determine the volume of gas between any two levels of mercury in the buret.

To fill the buret with a sample of gas requires special precautions in order to exclude the possibility of contaminating the sample with air. The sample is best introduced into the buret by the apparatus illustrated in Figure 2.

It includes a manifold, *A*, one end of which, *B*, is connected to a vacuum pump while the opposite end, which is equipped with a stopcock, *C*, is joined to a mercury blow-off, *D*, and to a gas cylinder, when such is the source of the sample. *A* branches at *K* into a parallel tube, *E*, which is connected by means of ground-glass joints, *F* and *G*, to the gas buret and to a receiver, *H*. The end of *E* carries a large hollow-barreled stopcock, *I*, which is sealed to a cap with a ground-glass lip fitting over a ground-glass container, *J*.

Procedure

When a gas sample is to be taken from a steel cylinder the latter is joined to the manifold, *A*, and the buret, after removal of its T-shaped head (*G*, Figure 1), is connected at *F*. After the system, including the buret, has been evacuated, *P* is closed and stopcock *C* set to allow but a small opening through which the gas is then allowed to enter the system while the surplus escapes through the mercury blow-off. More than the required quantity of hydrocarbon is condensed into the receiver, *H*, by cooling the latter in liquid air. *C* is then closed, the liquid air removed, and the pump allowed to run while the solidified hydrocarbon liquefies. In order to remove noncondensable impurities completely the process of alternate solidifying and liquefying of the gas under the action of the pump is repeated once or twice. After the final removal of the liquid air from *H*, *P* is opened, *K* is closed, and the hydrocarbon is allowed to distill into the buret until atmospheric pressure has been reached.

If the sample to be analyzed is in a sealed glass ampoule, *L*, the latter is placed in *J* and enough mercury added to bring the

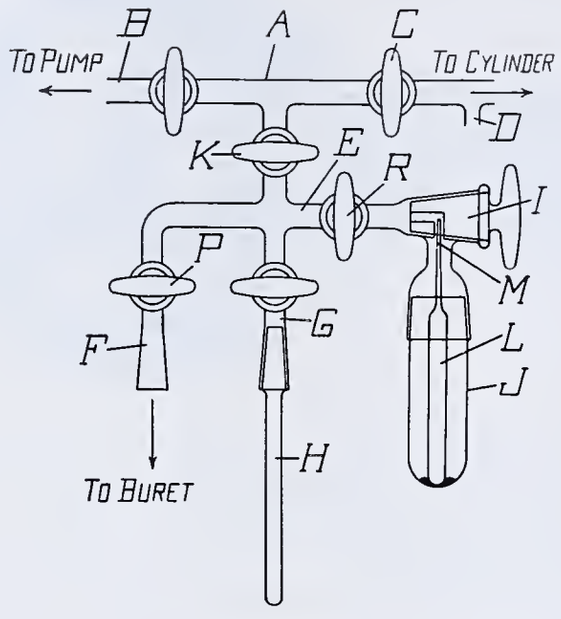

FIGURE 2

tip, M, of the ampoule into the bore of I. D is cut off at C and the system, including the buret, is evacuated. P is then closed, J is immersed in liquid air to condense the gas, and K is closed. After the gas has solidified, M is broken by a sharp turn of I. The liquid air flask is removed from J and raised around H and the gas is allowed to distill over and condense in H. Stopcock R is closed, K is opened, and the system is pumped while the hydrocarbon liquefies. As in the case mentioned above, the alternate process of freezing and pumping is repeated once or twice. Finally, the liquid air is removed, the system is cut off from the pump at K, P is opened, and the gas is allowed to distill into the buret until atmospheric pressure has been reached.

After removal of the buret from the filling apparatus the T-shaped head (G, Figure 1) is attached to it and the buret is inserted in the combustion train between J and I, as shown in Figure 1. The usual precautions prior to combustion are followed closely, except as outlined below. For the combustion of solids Pregl found that the optimum speed of the stream of oxygen going through the train as measured in the bubble counter should be 5 cc. per minute. In the case of gases it is essential to slow this down to 3 cc. per minute. It is also preferable to insert an empty platinum boat in the combustion tube and to keep it hot with the movable burner, exactly as for the combustion of solids.

To carry out the combustion the weighed absorption tubes are connected into the train, the flow of gas is adjusted, and the barometric pressure, temperature, and buret reading are recorded. The lower stopcock is then opened and the leveling bulb adjusted so that the mercury in B shows a head of 2 to 3 mm. The upper stopcock is now opened and the gas forced into the combustion tube by the gradual and careful raising of the leveling bulb at such a rate that the time required to introduce the sample is from 15 to 20 minutes, depending on the volume of gas. After the sample has been introduced into the combustion tube, the upper stopcock is closed, the level of the mercury adjusted, and the reading recorded. The stream of oxygen is kept up at the same speed for 5 minutes more and the sweeping operation is effected by a stream of air forced through the train at the usual rate of 5 cc. per minute. In the course of this sweeping operation 200 cc. of water are collected from the Mariotte flask in 40 minutes. After the products of the combustion have been so swept out from the combustion tube the absorption tubes are disconnected, wiped, and weighed as usual.

The volume of gas used in a combustion will obviously vary with the hydrocarbon and should be such as to correspond to a weight of 3.5 to 4.5 mg., as in the ordinary microanalytical technique.

From the weights of carbon dioxide and water the weights of carbon and of hydrogen in the sample of hydrocarbon are obtained and their sum is equal to the weight of the sample. From these the percentage composition is determined. Furthermore, from the weight of the sample thus obtained (sum of carbon and hydrogen) and the volume of hydrocarbon taken, corrected for normal temperature and pressure, the molecular weight is calculated.

This method of analysis has been applied to a series of saturated and unsaturated hydrocarbons and the results obtained are shown in Table I.

It will be seen on examining Table I that samples of hydrocarbons as small as 2 to 3 cc. may be analyzed and the carbon and hydrogen content determined with an accuracy of ±0.2 per cent.

TABLE I. ANALYSES OF HYDROCARBONS

Gas Sample	Volume (Corrected) *Cc.*	Wt. of Hydrogen *Mg.*	Wt. of Carbon *Mg.*	Hydrogen %	Carbon %	Mol. Wt.	Ratio of C to H
Ethane	...	...	...	20.00	80.00	30.00	4.00
	1.862	0.491	1.970	19.95	80.05	29.63	4.01
	2.263	0.599	2.427	19.79	80.21	29.95	4.03
Propane	...	...	...	18.18	81.81	44.00	4.50
	1.974	0.707	3.177	18.22	81.78	44.08	4.49
	1.981	0.705	3.192	18.11	81.89	44.08	4.52
Butane	...	...	...	17.24	82.76	58.00	4.80
	1.524	0.669	3.245	17.09	82.91	57.53	4.85
	1.392	0.625	3.000	17.25	82.75	58.37	4.80
Cyclopropane	...	...	...	14.29	85.71	42.00	6.00
	2.176	0.568	3.466	14.08	85.92	41.52	6.10
	2.112	0.564	3.398	14.24	85.76	42.03	6.02
Acetylene	...	...	...	7.69	92.31	26.00	12.00
	3.232	0.280	3.447	7.52	92.48	25.84	12.28
	3.138	0.278	3.374	7.63	92.37	26.08	12.10
Ethylene	...	...	...	14.29	85.71	28.00	6.00
	2.241	0.400	2.401	14.28	85.72	28.00	6.00
	2.186	0.394	2.338	14.42	85.58	28.00	5.94

TABLE II. ANALYSES OF ETHYLENE OXIDE

Volume (Corrected) *Cc.*	Wt. of Sample Calcd. from Mol. Wt. *Mg.*	Wt. of H_2O *Mg.*	Wt. of CO_2 *Mg.*	H_2 %	C %
...	...	...	...	9.09	54.54
1.986	3.901	3.152	7.774	9.04	54.35
1.939	3.808	3.122	7.589	9.17	54.34

With an unknown gas, the weight of the sample is obtained from the sum of the weights of carbon and hydrogen and thus the percentage composition will always add up to 100 per cent. This method, however, does not seem to involve any appreciable error, as evidenced by the results given in Table I, since in all cases, although the gas was known, the weight of the sample was obtained in this manner. The percentage composition of the gas does not always enable one to judge of its homogeneity since, for instance, an olefin contaminated with another olefin will have the same percentage composition as if it were homogeneous. The purity of the gas, however, can be judged from the molecular weight which can be calculated with an accuracy of ±0.5 gram, from the corrected volume and the sum of the weights of carbon and hydrogen. Since the calculation of the molecular weight involves both the weight of the sample and the corrected volume, the value obtained is affected by the presence of any impurity having a different molecular weight. For instance, butylene contaminated with ethylene will have the same percentage composition as pure butylene but a much lower molecular weight. The identity of the sample is based on the correlation of the molecular weight and the percentage composition.

This method can be applied to the analysis of gases containing oxygen as well as carbon and hydrogen, such as ethylene oxide (see Table II). In such cases the weight of the sample cannot be obtained from the analytical results. When dealing with a gas of unknown identity this difficulty could be overcome by determining the density of the gas by a micromethod prior to carrying out the analysis.

Acknowledgment

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Interferences Occurring with Selected Drop Reactions

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AS A GENERAL rule drop tests and other color reactions are developed especially for use for specific tests. There are, however, few truly specific reactions and there is need for information concerning such interferences as may occur and steps that may be taken to remove them. Systems of semimicroqualitative analysis have been developed (*1, 2, 8, 10*) in which spot tests are used for the identification of the different ions after the usual group separations. Gutzeit (*5*), Krumholz (*4*), and Heller (*6*) have proposed schemes of qualitative analysis based on the more simple separations. The interests of the authors have been more closely allied to those of the latter group of workers, and a system of analysis has been developed (*9*). Special consideration has been given to the adaptation of this system to use in portable kits.

In the system employed by the authors, a two-group separation of the elements under consideration is used. The first step is either fusing the sample with sodium carbonate and sodium peroxide, or adding sodium carbonate and then sodium peroxide to a solution of the unknown. In either case, the water-soluble carbonates and hydroxides are separated from the insoluble oxides, carbonates, hydroxides, etc., by filtration. This accomplishes an almost equal division of the more common elements into two major groups. One of the main advantages of this procedure is that oxidation by sodium peroxide yields the elements in a constant state of oxidation, usually the highest. This adjustment of valence has two main advantages: (1) since all forms of an element are converted to one common valence, many tests are eliminated; (2) the elimination of extra valence forms reduces the number of possible interferences.

When this procedure was first investigated, no study of interferences was made. Instead, the interferences listed by Feigl (*3, 4*) were noted, and the effect of the proposed separation procedure in eliminating these interferences was taken into account. Throughout 4 years of observation and use the two-group separation has proved to be of great value in conducting microchemical analyses by means of drop tests. In the course of this work it has been observed that there are a considerable number of interferences not listed in the general literature on drop reactions. For this reason the investigation on which the present report is based was undertaken.

Solutions and Reagents

For the study of interferences, solutions were made up to contain approximately 5.0 mg. per ml. of each element to be studied. In the preparation of each such solution, an appropriate amount of the C. P. chemical was fused in a platinum crucible with sodium carbonate and sodium peroxide. The melt was then dissolved in water, the pH was adjusted to a value as near 7.0 as possible, and the solution was boiled to decompose the remaining hydrogen peroxide. After being cooled, the solutions were made up to the required volume and placed in convenient Pyrex dropping tubes.

By this procedure, solutions were prepared for lithium, sodium, potassium, copper, silver, gold, beryllium, magnesium, calcium, zinc, strontium, cadmium, barium, mercury, boron, aluminum, carbon, silicon, titanium, zirconium, tin, lead, thorium, nitrogen, phosphorus, vanadium, arsenic, antimony, bismuth, sulfur, chromium, selenium, molybdenum, tungsten, uranium, fluorine, chlorine, manganese, bromine, iodine, iron, cobalt, and nickel. Only one solution was needed for each element, irrespective of the number of variations in valence in which it occurs, since the action of the sodium peroxide in the preparation of the solution leaves each element (except manganese) in a single state of oxidation, usually the highest. In the case of manganese, a fresh known solution was made up each day, since the solution gradually decomposed to give variable mixtures of manganese dioxide, manganates, and manganous salts.

The reagents and apparatus used in this work have been described previously (*9*).

Method of Studying Interferences

The actual investigation of interferences was carried out according to a definite scheme. Because of the varied forms of phosphate interference met with, special attention was paid to the behavior of this ion under each set of conditions studied. The following series of solutions was prepared and used in studying the interferences with each test.

1. Blank
2. Element under consideration
3. Element plus phosphate
4. Individual members of periodic group I, in the absence of the element to be studied
   Individual members of periodic group II, in the absence of the element to be studied, etc.
5. Individual members of periodic group I plus element
   Individual members of periodic group II plus element, etc.
6. Individual members of periodic group I plus phosphate, in the absence of the element
   Individual members of periodic group II plus phosphate, in the absence of the element, etc.
7. Individual members of periodic group I plus phosphate plus element
   Individual members of periodic group II plus phosphate plus element, etc.

The interferences found were reported as positive if a false test was obtained in the absence of the element sought, and negative if the test failed to indicate presence of the element

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TABLE I. ANALYTICAL PROCEDURES AND FINDINGS

Element	Procedure	Interferences Listed: Feigl (3, 4)	Interferences Listed: Lange (7)	Interferences Found[a]	Remarks
		Aqueous Group			
Al	Spot plate 1. 1 drop of soln. 2. 1 drop of 0.1 *N* NaOH 3. 1 drop of alizarin S 4. 2 drops of acetic acid Orange color	Fe, Cu, Co, alkaline earths, Cr	Fe, Cr, Co, Mn	Cu(+), Ag(+), Ba(+)[b], Zr(+), Pb(+), Th(+), V(+), Bi(+), Mo(+), Cr(+), Fe(+), Ni(+), Co(+), Ca(+), Be(+)	Test drop should be alkaline prior to addition of alizarin S. Final pH should be less than 4.0. After Na_2CO_3-Na_2O_2 separation. V, Mo, and Cr are only interfering elements present.
Sb	A. Watch glass 1. 2 drops of soln. 2. 1 drop of $(NH_4)_2S$ 3. 1 drop of concd. HCl 4. Remove excess liquid 5. 1 drop of concd. HCl Warm B. Filter paper 1. 1 drop of phosphomolybdic acid 2. 1 drop of soln. A 3. Hold in steam Blue stain	Sn^{++}	Not listed	Sn^{++}(+)	$(NH_4)_2S$ separation yields Sn in stannic condition which does not interfere, thus making test specific.
As	Gutzeit apparatus A. Place in body of apparatus 1. A few grains of Zn 2. 1 ml. of 0.1 *N* HCl 3. 4 drops of $SnCl_4$ 4. 2 to 4 drops of test soln. 5. Put reaction stopper in place B. Reaction stopper should be filled with PbAc paper and covered with $HgBr_2$ paper. Yellow to black stain	Sb, S^{--}, Hg, PO_4^{---}	Not listed	Au(−), Ag(−), Ba(−), Hg(−), Pb(−), Bi(−)	Interfering ions slow up reaction but by adding fresh Zn at intervals and allowing reaction to proceed 20 minutes or more test is specific for qualitative work.
Be	Casserole 1. 1 drop of soln. 2. 1 drop of concd. HCl 3. Evap. to dryness 4. Dissolve in 1 drop 5 *N* NaOH 5. 1 drop of quinalizarin Blue color	NH_4^+, Cu, Ni, Fe, Mg, tartrates in presence of aluminum	Fe, PO_4^{---}, Mg	Ag(−), Au(−), Cu(−), Mg(+), Cd(+), Ba(+), NH_4^+(+)	1 drop of KCN will mask Cu, Ag, and Au interferences. 1 ml. of Br_2 water fades color due to Mg but not that due to Be. Test is inconclusive in presence of Cd and Ba.
B	Glass spot plate 1. 1 drop of turmeric 2. 1 drop of soln. 3. 1 drop of 0.1 *N* HCl 4. Evaporate to dryness 5. 1 drop of 5 *N* NaOH Greenish-blue color (momentary)	Oxidizing agents, I^-	PO_4^{---}, SiO_3^{--}, oxidizing agents	PO_4^{---}(−), Au(+), Be(−), Si(−), Zn(−), Ti(−), V(−), Cr(−), Mo(−), Fe(−), Co(−)	Many ions give red colors with turmeric but only B and Au stains turn blue upon addition of NaOH. Na_2CO_3-Na_2O_2 separation eliminates all interferences except those due to PO_4^{---}, Si, Be, V, Cr, and Mo, all of which tend to inhibit test
Cl	Chloride apparatus A. Place in ignition tube of apparatus 1. Suitable amount of dry sample 2. Few grains of K_2CrO_4 3. 1 drop of concd. H_2SO_4 B. Place 1 drop of fresh diphenylcarbazide in reaction capillary C. Warm ignition tube Pink color in reaction capillary	Ag, Hg, NO_2^-, Br, I, F	Not listed	Ag(−), Hg(−), I(−), Br(−), F(+)	Test is inconclusive in presence of Ag, Hg, and F. Very large amts. of I and Br mask test.
Cr	Spot plate 1. 1 drop of soln. 2. Acidify (H_2SO_4) 3. 1 drop of diphenylcarbazide Pink color	Hg, V, Mo	Fe does not interfere	Hg(+), Zr(+), V(+), Mo (blue), Fe(+)	V and Mo are only interfering elements appearing in aqueous group. Mo interference may be eliminated by adding 1 drop of oxalic acid to test drop.
F	Spot plate 1. 1 drop of soln. 2. 1 drop of 0.1 *N* HCl 3. 1 drop of Zr-alizarin soln. Pink reagent fades to yellow	SO_4^{--}, $S_2O_3^{--}$, PO_4^{---}, AsO_4^{---}, $C_2O_4^{--}$	SO_4^{--}, PO_4^{---}	Be(−), Al(−), Si(−), Th(−), Sb(−), S(+), Cr (masks)	Zr and Th appear in other group. S (SO_4^{--}) may be removed by adding 1 drop of $BaCl_2$ and filtering off $BaSO_4$ ppt. Be, Al, Si, and Sb suppress test if present in large amounts.
I	Spot plate 1. 1 drop of soln. 2. 1 drop of 0.1 *N* HCl 3. 1 drop of fresh starch paste (cold) 4. 1 drop of KNO_2 Blue color	Test inhibited by alum, $MgSO_4$, alkali sulfate, cyanides and certain organic compounds	Interferences not mentioned	Ag(−), Hg(−), Pb(−)	Specific within acid group.
Mo	Filter paper 1. 1 drop of concd. HCl 2. 1 drop of soln. 3. 1 drop of KCNS 4. 1 drop of $SnCl_2$ Red stain on edge of spot (not faded by $SnCl_2$)	Sensitivity reduced by HCHO, H_3PO_4, organic acids, Hg salts, nitrites	Not listed	Au (red color), Se(+), Fe(+), W (blue stain)	Stain due to Au turns purple when $SnCl_2$ is added. Red stain due to Mo is carried past any blue Au or W stain by capillary action. This capillary movement of Mo stain also differentiates it from red Se stain which is insoluble in HCl and therefore remains at center of spot. A specific test.
P	Spot plate 1. 2 drops of $(NH_4)_2MoO_4$	Si, As	Interferences not given	Si(+), As(+)	All interferences are eliminated by adding 1 drop of concd. HCl, evaporating to dryness, and extracting with HNO_3.

TABLE I (*Continued*)

Element	Procedure	Interferences Listed: Feigl (*3, 4*)	Interferences Listed: Lange (*7*)	Interferences Found[a]	Remarks
			Aqueous Group(*Con't*)		
(*Con't*)	2. 1 drop of soln. 3. 2 drops of HNO_3 4. 1 drop of $SnCl_2$ Blue color				Extract will contain only PO_4^{---} as As distills off as chloride and Si is converted to insoluble SiO_2. Si interference alone may be prevented by adding small crystal of tartaric acid to $(NH_4)_2MoO_4$ drop.
K	Spot plate (black) 1. 1 drop of soln. 2. Evaporate to dryness 3. 1 drop of acetic acid 4. Few grains of sodium cobaltinitrite Yellow ppt.	Li, Tl, NH_4^+	Not listed	Pb(+) soluble when heated. Li(+)	Lithium interferes. Lead gives yellow ppt. soluble when heated, thus differentiating it from Li ppt.
Se	Spot plate 1. 1 drop of soln. 2. 1 drop of thiourea 3. 1 drop of HNO_3 Red color	Cu, NO_3^-, Te (yellow ppt.). Bi (yellow ppt.)	NO_3^-, Cu	PO_4^{---}(−), N(−), Au (brown ppt. soluble in excess reagent), Cu(−), V (very light blue color, does not interfere), Bi (yellow color), As(−). Sb(−), Cr (blue, green, or brown color. Very large amounts mask the test)	Only interfering element in this group is Cr.
Si	Casserole 1. 2 drops of $(NH_4)_2MoO_4$ 2. 1 drop of soln. 3. Warm, cool, and filter from any visible ppt. of $(NH_4)_3PO_4 \cdot 12MoO_3$ 4. 2 drops of oxalic acid 5. 1 drop of $SnCl_2$ Blue color	AsO_4^{---}	PO_4^{---}, AsO_4^{---}	As(+)	Arsenic may be removed by evaporating test drop in presence of 1 drop of concd. HCl.
Na	A. Watch glass 1. 2 drops of soln. 2. 1 drop of $(NH_4)_2S$ 3. 1 drop of $(NH_4)_2CO_3$ 4. Filter B. Spot plate (black) 1. Filtrate A 2. 2 drops of acetic acid 3. 6 to 8 drops of zinc uranyl acetate Yellow ppt. (allow 5 minutes to form)	None, except in very concentrated solns.	PO_4^{---}, proteins, large amounts of K	Ti(−), Pb(−), As(+), PO_4^{---} (white ppt.)	Sulfide separation removes As and Pb. Large amounts of Ti reduce sensitivity. PO_4^{---} interferes, owing to masking effect of zinc phosphate ppt.
S	Casserole 1. 1 drop of soln. 2. 1 drop of HNO_3 3. 1 drop of $Hg(NO_3)_2$ 4. Evaporate to dryness 5. 2 drops of H_2O Yellow ppt.	None	Not listed	V(+), Sb and As gave ppts. which masked test, Cr (masks)	If V, Sb, As, or Cr is present alternate procedure such as $BaCl_2$ test (carried out on black spot plate) should be used.
Sn	A. Watch glass 1. 1 drop of soln. 2. 1 drop of $(NH_4)_2S$ 3. Absorb excess liquid 4. 1 drop of concd. HCl 5. Add few grains of powdered Mg and heat until Mg dissolves B. Filter paper 1. 1 drop of soln. A 2. 1 drop of phosphomolybdic acid Blue stain	Sb	Not listed	Sb(+)	If Sb is absent proceed from B. Test is specific.
W	Filter paper 1. 1 drop of concd. HCl 2. 1 drop of soln. 3. 1 drop of KCNS 4. 1 drop of $SnCl_2$ Blue stain in center of spot	Mo, H_3PO_4, certain organic hydroxy acids	Not listed	Au (red stain changing to blue upon addition of $SnCl_2$), Mo (red stain on edge of drop), Fe (red stain; disappears upon addition of $SnCl_2$), Se (red color in center of spot)	Specific when attention is paid to color and position of stains. Au and W both give blue stains in center of spot but Au gives a preliminary red stain upon addition of KCNS while W does not.
V	Filter paper 1. 1 drop of aniline 2. 1 drop of HNO_3 3. 1 drop of soln. 4. Warm Green stain	Not listed	Not listed	PO_4^{---}(−), Sn(−), Cr(+), Br (yellow-brown color), I(+)	Cr, Br, I, Sn, and large amounts of PO_4^{---} interfere. Confirm by 1 drop of soln., 1 drop of concd. H_2SO_4, 1 drop of H_2O_2. Red color.
			Acid Group		
Ba	A. Watch glass 1. 2 drops of soln. 2. 1 drop of $(NH_4)_2S$ 3. Filter B. Filter paper 1. 1 drop of filtrate A 2. 1 drop of 0.1 *N* HCl 3. 1 drop of sodium rhodizonate (fresh) 4. Let stand until color develops 5. 1 drop of 0.1 *N* HCl brown-red stain; HCl causes slow fading	Heavy metals, Sr	Sr	PO_4^{---}(slight −), Sr (red stain; faded by HCl), Pb (red stain; turns purple upon addition of HCl), As(−), Sb(−), Cr(−), Fe (green-blue stain)	Sr stain differentiated from Ba stain since it fades quickly upon addition of HCl. All* other possible interferences are eliminated by pptn. as sulfides.

(*Continued on pages 274 and 275*)

TABLE I (*Continued*)

Element	Procedure	Interferences Listed Feigl (3, 4)	Lange (7)	Interferences Found[a]	Remarks
			Acid Group (*Con't*)		
Bi	Spot plate 1. 1 drop of soln. 2. 1 drop of thiourea 3. 1 drop of HNO_3 Yellow color	Not listed	Specific	PO_4^{---}(−), Au (brown ppt., soluble in excess reagent), V (blue color), As(−), Sb(−), Cr (masks), Se (red ppt., masks)	Specific. All possible interferences appear in aqueous group.
Cd	Spot plate 1. 1 drop of diphenylcarbazide thiocyanate 2. 1 drop of soln. 3. 1 drop of sodium acetate 4. 1 drop of NH_4OH Violet-pink color	Only very large amounts of Hg, Cu, Pb, and Bi interfere	Specific within Group II	PO_4^{---} (slow up reaction), Cu(+), Ag(+), Au(+), Hg(+), V (blood red color), As(−), Sb(−), Cr(+), Fe(+), Pb (purple color), Bi(+), Ni(+), Co(purple color)	All interferences except Cu and Fe are eliminated by Na_2CO_3–Na_2O_2 separation Fe reduced to ferrous state does not interfere. Cu does not interfere if crystal of KI is added to reagent prior to test.
Ca	Watch glass 1. 2 drops of soln. 2. 1 drop of $(NH_4)_2S$ 3. Warm, filter Spot plate (black) 1. Filtrate A 2. 1 drop of fresh saturated soln. of dihydroxy tartaric osazone Yellow precipitate	Only alkali and ammonium salts may be present	Alkaline earth metals	Li(+), Cu(+), Au(+), Ag(+), Be(+), Zn(+), Sr(+), Ba(+), Al(+), Zr(+), Sn(+), Pb(+), Th(+), V(+), As(+), Sb(+), Bi(+), Mo(+), Mn(+), Fe(+), Ni(+), Co(+)	All interferences are removed except those due to Be, Sr, and Ba. Very large amounts of Mg may give false test.
Co	Filter paper 1. 1 drop of soln. 2. 1 drop of α-nitroso-β-naphthol 3. Hold over NH_4OH Brown stain	Fe, U, Cu, Pd	None listed	Cu(+), Au(+), Zr(+), V(+), U(+), Cr(+), Fe(+)	V and Cr appear in aqueous group. Zr, Cu, U, and Fe interferences may be prevented by adding 1 drop of H_3PO_4 and 1 drop KI to 1 drop of soln. on watch glass, stirring, then adding 2 drops of $Na_2S_2O_3$. Test on resulting soln. is specific.
Cu	Casserole 1. 1 drop of soln. 2. 1 drop of $(NH_4)_2HPO_4$ 3. Acidify (concd. H_2SO_4) 4. 1 drop of $ZnSO_4$ 5. 1 drop of ammonium mercuric thiocyanate Violet ppt.	Fe, Cd, Co, Ni	Not listed	Au (brick-red ppt., masks), Cd (powder-blue ppt.), Fe (red color), Co (green ppt.) Ni (green-blue ppt.)	Gold is only interference if a control is run since colors due to other elements do not mask nor resemble positive Cu test.
Au	Filter paper 1. 1 drop of soln. 2. 1 drop of $SnCl_2$ 3. 1 drop of KCNS 4. Boil in water Purple stain	Not listed	Not listed	W(+)	Stain given by W is distinguished from true test for Au by fact that W stain fades in boiling water while Au stain does not.
Fe	Spot plate 1. 1 drop of soln. 2. 1 drop of KCNS 3. 1 drop of 0.1 *N* HCl Red color	PO_4^{---}, organic hydroxyl compounds, Hg, NO_2^-	Not listed	PO_4^{---}(−), Cu (slight yellow color), Au(+), Hg(−), Ag(−), As(−), Cr (masks), Mo(+), F(−), Mn (masks)	Na_2CO_3–Na_2O_2 separation removes interferences due to PO_4^{---}, Hg, Ag, Cr, Mo, and F. As, oxalates, citrates may be removed prior to test by evaporating in presence of 1 drop concd. HCl and roasting.
Pb	Spot plate 1. 1 drop of soln. 2. 2 drops of KCN 3. 1 drop of NH_4Cl 4. 1 drop of dithizone 5. Compare at once with blank Pink color in CCl_4 layer	Specific	Sn^{++}, Bi, Tl	Sb(+), Bi(+)	Specific if blank and control are run, since Sb gives off-color green-brown soln. Bi gives red color similar to that given by Pb.
Mg	A. Watch glass 1. 2 drops of soln. 2. 1 drop of $(NH_4)_2S$ 3. Warm and filter B. Spot plate (white) 1. 1 drop of soln. A 2. 1 drop of KCN 3. 1 drop of Titan yellow 4. 1 drop of 5 *N* NaOH Red color	Al, Zn, Sn, Cd, Ni, Co	Al, Ni, Co, Mn, Zn, Sn, NH_4^+	Au(−), Zn(+), Cd(+), Hg(−), Al(−), Mn(+), Fe(+), Ni(+), Co(+), Sn(+)	Test is specific when Na_2CO_3–Na_2O_2 and $(NH_4)_2S$ separations are used.
Mn	Casserole 1. Portion of original sample 2. 1 to 4 drops of $AgNO_3$	Cl, I, Br	Not listed	Cl(−), Br(−), I(−)	Test is specific since interference due to halogens may be prevented by adding excess $AgNO_3$.

when the element was actually present. Table I summarizes the analytical procedures used and the findings of the investigation.

Conclusions

A systematic study of interferences occurring with selected drop reactions has been made. The investigation dealt with tests for 38 elements.

Special attention is given to the types of interferences encountered and the term "positive interferences" is applied to interferences giving false tests. Interferences which inhibit a true positive test are designated by the term "negative interference".

The data obtained in this investigation are tabulated and applied to a study of the two-group sodium peroxide–sodium carbonate system of analysis. The effect of this separation on the elimination of interferences is noted.

TABLE I (*Concluded*)

Element	Procedure	Interferences Listed: Feigl (3, 4)	Interferences Listed: Lange (7)	Interferences Found[a]	Remarks
			Acid Group (*Con't*)		
Mn (*Con't*)	3. 1 drop of H_3PO_4 4. Few grains of $(NH_4)_2S_2O_8$ 5. Warm Pink color				
Hg	Spot plate 1. 1 drop of soln. 2. 1 drop of sodium acetate 3. 1 drop of dimethylaminobenzal rhodanine 4. Run blank Pink color	Cl, Cu	Ag	Au(+), Ag(+), Cu(+), Si(−), Cl(−)	pH of test soln. should be between 3 and 7. Acetic acid makes best medium. When blank is run Cu and Au are only interferences. Interferences due to Cu may be prevented by adding 1 drop of Na_2HPO_4 to test drop.
Ni	A. Watch glass 1. 1 drop of soln. 2. 1 drop of H_2O_2 3. 1 drop of $(NH_4)_2CO_3$ 4. 1 drop of NH_4OH B. Filter paper 1. Absorb soln. A on filter paper. 2. 1 drop of dimethylglyoxime Pink stain	Pd, Pt, Co, Fe^{+++} + Co, Cu, Mn, Fe^{++}	Fe^{++}, Co, Pd	Mn(−), Fe(+), Co(+)	Test is specific when preliminary treatment A is carried out.
Ag	Spot plate 1. 1 drop of acetic acid 2. 1 drop of K_2CrO_4 3. 1 drop of test soln. containing $(NH_4)_2CO_3$ Red ppt.	None given	Not listed	MnO_4^- (masks)	MnO_4^- may be reduced with 1 drop of thiourea to $MnO_2.xH_2O$ and filtered off, making test specific.
Sr	A. Watch glass 1. 2 drops of soln. 2. 2 drops of $(NH_4)_2S$ B. Filter paper 1. Filtrate from A 2. 1 small drop of 0.1 *N* HCl 3. 1 drop of K_2CrO_4 4. 1 drop of sodium rhodizonate Brown stain	Ba, heavy metals	Ba	Ag (masks), Pb(+), As(−), Sb(−), Fe(−)	Test drop should have pH between 5 and 7. Barium interference is prevented by pptn. as $BaCrO_4$. Other interferences are removed by sulfide pptn.
Ti	Spot plate 1. 1 drop of soln. 2. 1 drop of H_3PO_4 3. 1 drop of H_2O_2 Yellow color	F, Cl, Br, I, V, Cr, Fe, Acetate	Not listed	V(+), Cr (blue color), Mo(+), F(−), Cl(−), Br(−), I (masks)	All interfering substances appear in aqueous group, making test specific.
U	A. Watch glass 1. 1 drop of soln. 2. 4 drops of $Na_2S_2O_3$ B. Filter paper 1. Filtrate from A absorbed on filter paper 2. 1 drop of $K_4Fe(CN)_6$ 3. 1 drop of 0.1 *N* HCl Brown stain	Specific	Not listed	Be (blue stain), V (yellow stain), Fe (green stain)	Other stains are so light that test may be considered specific.
Zn	Casserole 1. 1 drop of soln. 2. 1 drop of $(NH_4)_2HPO_4$ 3. 2 drops of H_2SO_4 4. 1 drop of $CuSO_4$ 5. 1 drop of ammonium mercuric thiocyanate 6. Warm Violet ppt.	Co, Fe, Ni	Not listed	Au (red ppt.), Cd (off-color blue ppt.), Fe (red color), Ni (green ppt.), Co (blue ppt.)	Test is specific if blank and control are run.
Zr	Glass spot plate 1. 1 drop of soln. 2. 1 drop of alizarin 3. Warm 4. 1 drop of HCl Brown-pink color	F, SO_4^{--}, PO_4^{---}, Mo, W, organic hydroxyacids	Not listed	PO_4^{---}(−), Si(−), Sb(−), W(−), Mo(−), F(−)	All interferences appear in aqueous group, making test specific.

[a] Interferences were studied without prior separations.
[b] −, negative interference (suppression of test). +, positive interference (false test).

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Studies of the Methoxyl Determination

Modification of Apparatus and Preparation of Hydriodic Acid

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IN THE course of an investigation, in which it was necessary to make a large number of methoxyl determinations, erratic results were encountered in running known samples, and were soon traced to the reagents. The importance of using highly purified reagents, especially hydriodic acid, is generally recognized (*6*, *10*); however, a review of the literature indicates that little has been published (*5*) regarding the nature of the interfering impurities or their probable origin. For this reason the following investigation was undertaken.

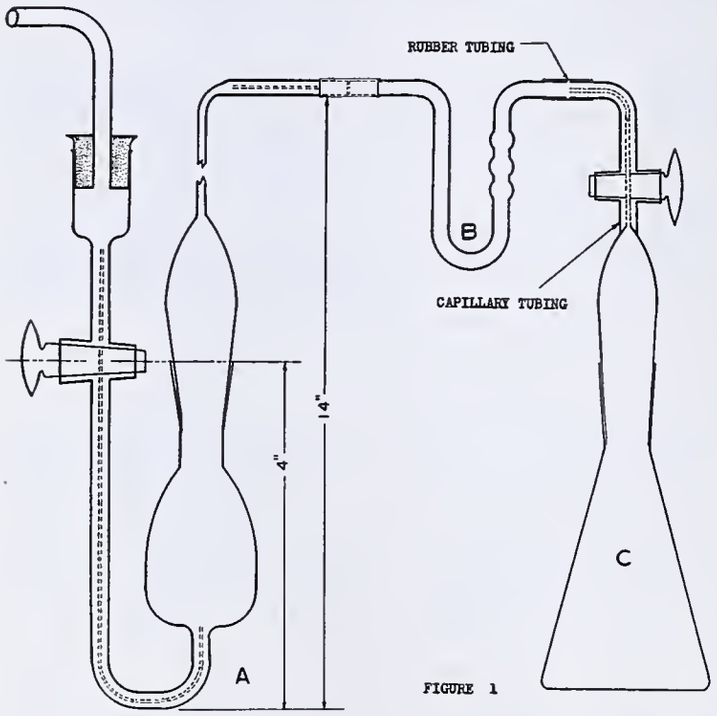

FIGURE 1

A modified Pregl apparatus was used. Many modifications (*1*, *2*, *3*, *15*, *16*, *17*) of the original methoxyl apparatus have been reported, and much attention has been given to the question of absorption (*7*, *9–12*, *15*, *18*). In this laboratory this difficulty was overcome by using an evacuated flask (containing the bromine solution) as a receiver for the alkyl iodide. Besides preventing loss of alkyl iodides, this arrangement provided an excellent control on the rate of gas flow.

Apparatus

The apparatus, made of Pyrex glass, is illustrated diagrammatically in Figure 1. The reaction unit, *A*, was constructed from a 14/35 standard taper joint and a capillary stopcock, and had a volume of 7 ml. to the ground-glass joint. The condensing unit, made from 5-mm. and capillary Pyrex tubing was 35 cm. over-all in length. It was equipped with a water jacket (not shown) and was connected to U-tube *B*, which in turn was connected to absorption flask *C*. The absorption flask for the analysis of the alkyl iodides was constructed from a 250-ml. Erlenmeyer flask, a 24/40 standard taper joint, and a two-way capillary stopcock.

A phosphoric acid bath (*4*) served as a heating medium for the reaction vessel. The temperature was maintained by a microburner within ±5° C.

Approximately 5 mg. of material weighed in a glass boat were introduced into the reaction chamber, along with a few crystals of phenol and 4 or 5 drops of acetic anhydride, and the vessel was connected to the air-condensing unit. The U-tube was removed, charged with 0.5 cc. each of 5 per cent cadmium sulfate and sodium thiosulfate solutions, and then connected to the apparatus.

The absorption flask was charged with 2 ml. of 10 per cent sodium acetate–glacial acetic acid solution and 5 drops of bromine. The joint was lubricated with glycerol and the flask was evacuated by means of a water pump and connected to the open arm of U-tube *B*.

After making sure that the charge had dissolved, 2 ml. of hydriodic acid were introduced through the capillary stopcock, which was then connected to an open Kipp generator for carbon dioxide. The gas flow was regulated to approximately 10 to 30 bubbles per minute (depending on time of heating) and the reaction vessel brought to a temperature of 120° to 130° C. by means of a phosphoric acid bath.

After the heating operation (from 30 to 60 minutes) the flask was disconnected and the male joint sufficiently opened to permit the pipetting of 5 ml. of 20 per cent sodium acetate solution. The joint was again closed, and by inverting and shaking the flask, the entire inner surface was washed with solution. Formic acid was added dropwise until the solution was colorless, 4 to 6 drops in excess being used. Two milliliters of 10 per cent potassium iodide were introduced, and the solution was acidified with 5 ml. of 2 *N* sulfuric acid. The liberated iodine was then titrated with standard thiosulfate. From these data the methoxyl content was readily calculated.

TABLE I. BLANK RUNS

Hydriodic Acid Sample	Blank, 0.02 *N* Thiosulfate *Ml.*
From H_3PO_4	
1	0.04
2	0.04
3	0.06
Baker's, sp. gr. 1.5 (not redistilled)	0.05
After first redistillation (ordinary distilling unit)	0.56
After second redistillation (same unit)	1.24
After third redistillation (all-glass unit)	0.08

TABLE II. PRECISION OF APPARATUS

	Sample Weight *Mg.*	Methoxyl Found %	Methoxyl Calculated %
Anisic acid	3.176	20.6	20.4
	4.380	20.3	
Dibromo-*p*-dimethoxybenzene	5.665	21.3	21.0
	4.159	21.1	
Vanillin	2.050	20.1	20.4
	3.030	20.2	
	2.775	20.1	
	4.048	20.2	
	3.731	20.3	

Discussion and Results

Blank runs, made with hydriodic acid obtained from various sources, gave values varying from 0.04 to 1.5 cc. of 0.02 *N* thiosulfate. Initial experiments indicated that the magnitude of the blank was due mainly to the hydriodic acid used. It was thus evident that the interfering impurities must be both volatile and readily converted to an oxidizing agent by the bromine—probably arsenic compounds or alkyl iodides.

A Gutzeit determination showed the presence of only traces of arsenic. To prove the presence of alkyl iodide as an interfering impurity in the hydriodic acid, the gases from a blank determination (using carbon dioxide–free air as a carrier gas) were passed over a heated platinum spiral. Both iodine and carbon dioxide were found in sufficient quantities to account for most of the blank. These experiments gave

reasonable assurance that a volatile organic iodide must have been present in the hydriodic acid. Clark appears to have recognized this fact when he suggested refluxing in a stream of carbon dioxide as one step in preparation.

The nature of the impurities in hydriodic acid depends, of course, on the reagents used in making it. Various methods are reported for its preparation (*5*, *8*, *13*, *19*), some involving the use of organic materials. In order to avoid either sulfur or organic contaminants, the authors prepared a number of samples of hydriodic acid by distilling a mixture of 85 per cent phosphoric acid and potassium iodide (*14*) under reduced pressure (in an all-glass distilling unit). Constant-boiling acid obtained from this source gave the values noted in Table I. This method of preparing hydriodic acid proved to be rapid and simple.

In connection with this work the deleterious effect of cork on purity of hydriodic acid was observed. To measure the relative effect of cork, a sample of hydriodic acid was distilled several times in an ordinary distilling unit provided with two cork stoppers. The importance of keeping hydriodic acid out of contact with organic matter such as cork is indicated in Table I.

To illustrate the precision of the modified apparatus, a number of successive runs with it are tabulated in Table II.

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Micro- and Drop-Scale Titration of Oxalate

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SMALL amounts of oxalate have been titrated in the determination of calcium and other materials as a routine part of many methods. The common procedures in which permanganate is employed without the use of an additional indicator become increasingly less dependable as the amount of oxalate is lowered, because of the combination of a feeble end-point color, a proportionally large blank, and a relatively low stability of both titrating solution and oxalate in dilute, hot, acid solution (*2*).

Rappaport and Rappaport (*5*) utilized excess ceric sulfate for the determination of small amounts of calcium oxalate, determining the excess iodometrically. Larson and Greenberg (*3*) recorded the use of a similar procedure in which ferrous ammonium sulfate was used to determine the excess ceric sulfate. They reported no detailed study of the conditions necessary for the titration. Lindner and Kirk (*4*) studied the factors influencing the use of this procedure on the drop scale, while Ellis (*1*) employed ammonium hexanitrato cerate in perchloric acid solution for the same scale of titration. The use of various cerates including nitrato and perchlorato cerate on a macro scale has been studied by Smith and Getz (*6*). In their work, the reagents were 0.05 *N*, which is considerably stronger than can be employed conveniently in the type of titration reported here. Since all cerate solutions appear to become less stable with dilution, this point is of importance.

Extended use of these oxalate titrations on the micro and drop scale in this laboratory has shown various discrepancies in and between the different procedures which require control, and each method appears to have definite limitations in range which have never been adequately defined. In this communication are reported a study and definition of the conditions and range for each of these titrations, and an investigation of the little studied field of the use of permanganate with indicators in micro- and drop-scale titrations.

Solutions

0.01 *N* Sodium Oxalate Solution, 0.1675 gram of pure sodium oxalate dissolved to make 250 ml. of solution.

Approximately 0.01 *N* Ceric Sulfate Solution. Approximately 6.7 grams of anhydrous ceric sulfate were heated gently with 28 ml. of redistilled sulfuric acid and 28 ml. of redistilled water. More water was added slowly to complete solution while warming, the volume being made to 400 to 500 ml. The solution was placed on a steam bath overnight and then diluted to 1 liter with redistilled water. The solution was standardized through ferrous ammonium sulfate (Mohr salt) solution against pure sodium oxalate, by the procedure of Smith and Getz (*6*).

0.01 *N* Mohr Salt Solution. Approximately 3.94 grams of pure ferrous ammonium sulfate were dissolved in approximately 0.1 *N* sulfuric acid to make 1 liter. This was standardized against pure sodium oxalate through ceric sulfate solution. This solution is relatively much more stable than the ferrous sulfate solution used by Smith and Getz.

0.01 *N* Hexanitrato Cerate in 1 *N* Perchloric Acid Solution, 20.21 grams of ammonium hexanitrato cerate dissolved in 200 to 300 ml. of water. To the solution were added 110 ml. of 60 per cent perchloric acid and water to make 1 liter. The resulting solution was heated on a steam bath for 24 hours, filtered, and standardized against pure sodium oxalate.

0.01 *N* Hexanitrato Cerate in 1 *N* Nitric Acid Solution, made as described above, except for the substitution of 65 ml. of concentrated nitric acid for the perchloric acid.

0.01 *N* Potassium Permanganate Solution. Approximately 3.5 grams of potassium permanganate were dissolved in water and diluted to 1 liter. This solution was allowed to stand for about 1 week and was filtered through asbestos which had previously been boiled in acid permanganate and washed. This solution, which was slightly more than 0.1 *N*, was carefully standardized against pure sodium oxalate and diluted as necessary to 0.01 *N* or 0.005 *N* with redistilled water. The diluted solution was in all cases rechecked against sodium oxalate solution.

Indicator Solutions. *o*-Phenanthroline–ferrous sulfate was prepared dissolving 1.485 grams of *o*-phenanthroline in 100 ml. of 0.025 *M* ferrous sulfate solution, to yield a 0.025 *M* stock solution. Small amounts of this solution were diluted with 9 parts of water before use. The indicator, either in stock or dilute solution, could be kept indefinitely. Setopaline C solution, made in 0.1 per cent aqueous solution, slowly deteriorated so that after a week it yielded a poor end point. It has been found in this laboratory that it can be regenerated as often as necessary by placing it on the steam bath for an hour with occasional shaking.

Table I. Conditions for Microtitration of Oxalate with Excess Ceric Sulfate and Mohr Salt

(5 ml. of 2 N H_2SO_4 added in all cases. 2.99 ml. of 0.0133 N $Ce(SO_4)_2$ used.)

0.0100 N Oxalate Taken	Titer, 0.0107 N Mohr Salt	Treatment
Ml.	*Ml.*	
None	3.706	Direct titration
	3.707	
	3.705	
1.003	2.826	30 minutes at room temperature
	2.811	
	2.825	
1.003	2.798	3 hours at room temperature
	2.801	
	2.803	
1.003	2.785	10 minutes on steam bath
	2.785	
	2.775	15 minutes on steam bath
	2.772	30 minutes on steam bath
	2.770	
	2.771	
	2.771	45 minutes on steam bath
	2.770	
	2.771	

Microtitrations with Ceric Sulfate

Since the determination of calcium rests directly on the titration of oxalate in the calcium oxalate precipitate, the titration of the oxalate in sodium oxalate solution with ceric sulfate was studied. Larson and Greenberg (*3*) implied that a short period of standing of the oxalate with excess ceric sulfate sufficed to complete the oxidation. Numerous trials of their procedure showed low and variable results. They did not employ a catalyst and in this study none is used, though the rate of the reaction of ceric sulfate with oxalate is increased markedly by use of iodine chloride or osmic acid. In Table I is shown the effect of different times of standing and of heat on the completeness of the reaction. To the sample was added a measured excess of ceric sulfate solution, the mixture was treated as indicated, and the excess cold ceric sulfate was titrated with standard ferrous ammonium sulfate solution, using *o*-phenanthroline–ferrous sulfate or Setopaline C as indicator. When no catalyst is used, the reaction does not reach completion unless the reactants are warmed on the steam bath for 30 minutes. The same length of time of standing in the cold gives about 98 per cent complete oxidation.

It is important that the acidity of the final solution be kept to at least 0.5 N and usually it was made about 1 N with sulfuric acid. At lower acidities several effects could be observed, all of which prevented an accurate determination. The usual first effect was a failure to observe a good color change, sometimes with fading. At still lower acidities hydrolysis of ceric sulfate was obtained with precipitation of the oxide.

The range of utility of the excess method with ceric sulfate is shown in Table II. By use of 0.005 N ceric sulfate it was found possible to determine the oxalate corresponding to as little as 0.02 mg. of calcium with an error in titration of not more than 1 per cent. Part I represents complete calcium analyses in which the errors arise from all sources inherent in the method. Parts II and III represent only the titration of sodium oxalate in accurately known amounts, the calcium equivalent being given in each case. In these parts, the errors listed represent only those of the titration itself. It is readily apparent that the titration performed in the manner specified involves errors which are usually negligible in comparison with those of the entire calcium determination, down to about 0.02 mg. of calcium, and using only standard microtechnique. Smaller quantities require drop-analysis methods (*4*) if an accuracy of about ±1 per cent is desired.

Microtitration with Hexanitrato Cerate

Ammonium hexanitrato cerate dissolved in 1 or 2 N nitric or perchloric acid (perchlorato cerate) may be used for direct titration of oxalate, owing to the high oxidation potential of these reagents (*6*) which causes an increased rate of oxidation. Perchlorato cerate was successfully used as a drop-scale reagent by Ellis (*1*). Application of these reagents for microtitration have been rare.

Sodium oxalate samples equivalent to the amounts of calcium indicated were acidified with nitric or perchloric acid and titrated directly with the corresponding cerate. Table III gives the results of the titration with nitrato cerate solution at two values of oxalate concentration. The data indicate a reasonable accuracy but are somewhat misleading in that the reaction was too slow for convenience and the end point (with *o*-phenanthroline–ferrous sulfate) was difficult to read, particularly in the presence of asbestos. The reaction was of about equal utility to the permanganate titration described below, but was slower.

The use of perchlorato cerate for direct titration of oxalate was considerably better, because of the satisfactory speed of oxidation. The sample was made about 1 N in perchloric acid and titrated in the cold with the perchlorato cerate solution, using Setopaline C as indicator. *o*-Phenanthroline–ferrous sulfate was less satisfactory, because of precipitation of the perchlorate of phenanthroline when the acidity was in excess of about 0.5 N. The end point with Setopaline C consisted in a final change from yellow to red, proceeding through a bronze which faded to yellow as the end point was approached. The final red color was permanent for several minutes and was readily visible in the presence of asbestos if about 30 seconds were allowed for full color development. Table IV shows a satisfactory accuracy in the titration with

Table II. Range and Accuracy of Oxalate Titration with Ceric Sulfate

Calcium Taken	Mohr Salt: Blank	Mohr Salt: Titer	Mohr Salt: Difference	Calcium Found	Error
Mg.	*Ml.*	*Ml.*	*Ml.*	*Mg.*	%
I. Calcium analysis, 4.96 ml. of 0.01 N ceric sulfate used					
0.540	5.959	3.415	2.544	0.545	0.9
0.540	5.959	3.406	2.553	0.546	1.1
0.540	5.959	3.412	2.547	0.545	0.9
0.526	5.963	3.507	2.456	0.525	0.2
0.526	5.963	3.512	2.451	0.526	0.0
II. Oxalate titrations, 2.99 ml. of 0.01 N ceric sulfate used					
Calcium Equivalent					
Mg.					
0.200	3.705	2.772	0.933	0.199	0.5
0.200	3.705	2.770	0.935	0.200	0.0
0.200	3.705	2.771	0.934	0.200	0.0
0.200	3.705	2.771	0.934	0.200	0.0
0.200	3.705	2.771	0.934	0.200	0.0
0.200	3.705	2.770	0.935	0.200	0.0
0.200	3.705	2.772	0.933	0.199	0.5
III. Oxalate titrations, 2.99 ml. of 0.005 N ceric sulfate used					
0.0199	3.686	3.334	0.352	0.0197	1.0
0.0199	3.686	3.333	0.353	0.0198	0.5
0.0199	3.686	3.333	0.353	0.0198	0.5

Table III. Microtitrations of Oxalate with Ammonium Hexanitrato Cerate

Ca Equivalent of Oxalate in Sample	Titer, 0.0085 N Cerate	Oxalate Found, Ca Equivalent	Error
Mg.	*Ml.*	*Mg.*	%
0.598	3.526	0.599	0.2
0.598	3.529	0.600	0.3
0.598	3.534	0.601	0.5
0.198	1.166	0.198	0.0
0.198	1.168	0.199	0.5
0.198	1.164	0.198	0.0

Table IV. Microtitrations of Oxalate with Ammonium Hexaperchlorato Cerate

Ca Equivalent of Oxalate in Sample	Titer, 0.0044 N Cerate	Oxalate Found, Ca Equivalent	Error
Mg.	*Ml.*	*Mg.*	%
0.200	2.287	0.201	0.5
0.200	2.274	0.200	0.0
0.200	2.285	0.201	0.5
0.200	2.282	0.201	0.5
0.200	2.288	0.201	0.5
0.200	2.282	0.201	0.5

oxalate corresponding to 0.2 mg. of calcium, but the method did not show itself to be so accurate as the back-titration using ceric sulfate oxidation, and was not found useful for the lower ranges of sample size.

Table V. Microtitration of Oxalate with Potassium Permanganate

Titer, $KMnO_4$	Blank	Oxalate Found, Calcium Equivalent	Error
Ml.	*Ml.*	*Mg.*	%
I. 0.0166 N $KMnO_4$ serving as its own indicator, oxalate equivalent to 0.200 mg. of Ca			
1.046	0.05	0.199	0.5
1.058		0.202	1.0
1.041		0.198	1.0
0.055		0.201	0.5
II. 0.0166 N $KMnO_4$, ferrous phenanthroline as indicator, oxalate equivalent to 0.200 mg. of Ca			
0.968	0.022	0.198	1.0
0.970		0.199	0.5
0.963		0.192	4.0
0.967		0.198	1.0
III. 0.0058 N $KMnO_4$, ferrous phenanthroline as indicator, oxalate equivalent to 0.200 mg. of Ca			
1.914	0.040	0.199	0.5
1.911		0.198	1.0
1.916		0.199	0.5
1.913		0.199	0.5
IV. 0.0116 N $KMnO_4$ Setopaline C as indicator, oxalate equivalent to 0.200 mg. of Ca			
0.906	0.006	0.200	0.0
0.899		0.198	1.0
0.898		0.198	1.0
0.898		0.198	1.0
0.900		0.199	0.5
0.894		0.197	1.5
0.896		0.198	1.0
V. 0.005 N $KMnO_4$, Setopaline C as indicator, oxalate equivalent to 0.040 mg. of Ca			
0.399	0.009	0.039	2.5
0.419		0.041	2.5
0.398		0.039	2.5
0.410		0.040	0.0
0.400		0.039	2.5

Drop-Scale Titration with Perchlorato Cerate

Many attempts to utilize perchlorato cerate for direct drop-scale titration of oxalate were unsatisfactory. In most of these the indicator was either precipitated (phenanthroline) or consumed by the oxidant (Setopaline C). If the end point was exactly known and the indicator added directly before reaching it, good results were obtained. Since this was impractical with unknowns, it was concluded that the method was not successful in the authors' hands. This is contrary to the experience of Ellis and probably indicates some differences in reagents or technique. Confirmation of Ellis' method (*1*) by other laboratories may be expected to clarify the discrepancy. No such difficulties have been encountered in the method of Lindner and Kirk (*4*), which is being used successfully in various laboratories including that of the authors.

Direct Microtitration with Permanganate

The use of permanganate for titrating small amounts of oxalate has long been a standard procedure but suffers from a progressively less distinct end point as the amounts are reduced. Internal indicators to sharpen the end point have not been much employed and seemed to offer an improvement. Since indicators such as *o*-phenanthroline–ferrous sulfate and Setopaline C are not stable in hot acid solution, a little manganous sulfate had to be added as a catalyst. The resulting cold catalyzed permanganate titration was definitely more precise than the usual hot titration and was probably more accurate as well, since the known errors of the hot titration (*2*) were eliminated. *o*-Phenanthroline–ferrous sulfate was not a very favorable indicator, because of its change from red to light blue and the obscuring of the latter color by the purple

Table VI. Drop-Scale Titrations of Oxalate with Potassium Permanganate

Calcium Equivalent of Oxalate Taken	Titer, 0.0213 N $KMnO_4$	Oxalate Found, Calcium Equivalent	Error
γ	λ	γ	%
9.57	22.40	9.48	0.9
9.57	22.49	9.56	0.5
9.57	22.40	9.48	0.9
7.23	16.97	7.18	0.7
7.23	16.90	7.15	1.1
7.23	16.85	7.13	1.4
5.49	12.92	5.46	0.5
5.49	12.87	5.44	0.9
5.49	12.90	5.45	0.7

of the permanganate. It could be used if added immediately before the end point, followed by very careful permanganate additions. Setopaline C was found satisfactory, but could not be added until the slow disappearance of the permanganate indicated approach to the end point. The color change was from blue green through lemon yellow to bronze, the latter change being most readily observed and taken as the end point. It was easily possible to use permanganate as dilute as 0.005 N.

Table V shows the results of titration with two strengths of permanganate, using both *o*-phenanthroline–ferrous sulfate and Setopaline C as indicators and permanganate as its own indicator. The possible accuracy was not greatly different in the three cases, but the blank values with permanganate as its own indicator were largest by a considerable amount, those with Setopaline C being least. The ease of titration was definitely greatest with the latter indicator. The permanganate titration was inferior to the excess ceric sulfate method, even when an internal indicator was used, because the accuracy could not be retained in the lower ranges of oxalate concentration.

Direct Drop-Scale Permanganate Titrations

Direct titration of oxalate with permanganate on the drop scale could best be done with *o*-phenanthroline–ferrous sulfate as the indicator, since in this case it was stable enough to be added at the start of the titration while Setopaline C was not. The permanganate was so diluted on this scale that it did not interfere with the perception of the blue end point.

Varying amounts of 0.01 N sodium oxalate were titrated with 0.0213 N potassium permanganate. In order to avoid indicator blank, the *o*-phenanthroline–ferrous sulfate was titrated exactly to its end-point color and the necessary amount was then added directly to the oxalate solution to be titrated. In measuring the sample an amount of indicator proportional to the amount of oxalate was always taken. In performing calcium analyses, the indicator would have to be added separately, but there could be no indicator blank in either case because of the preliminary adjustment of the indicator to its end-point color. After addition of manganous sulfate solu-

tion as a catalyst, the titration was performed in the cold. Table VI shows the accuracy and precision of such titrations on the drop scale. Permanganate used in this manner is a satisfactory oxidant for drop-scale use, but was not found to be so favorable for this purpose as the excess ceric sulfate method previously studied (*4*).

Summary

A comparative study of micro- and drop-scale titrations of oxalate and of calcium determination was made with excess ceric sulfate, ammonium hexanitrato cerate, ammonium hexaperchlorato cerate, and potassium permanganate solutions as reagents.

The necessary conditions for the titration were defined and the range, accuracy, and general utility studied for each titration.

The excess ceric sulfate method was found to have the widest range of applicability for both micro- and drop-scale titrations, and to be capable of the greatest accuracy.

Both ammonium hexaperchlorato cerate and potassium permanganate could be used for direct microtitrations of oxalate, but only the latter was useful for drop-scale work. Improvement in the use of permanganate was achieved by titrating the cold solution, using an internal indicator and manganous salt as a catalyst.

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Determination of Dietary Fluorine

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IN A STUDY of physiological action of ions of sea water the presence of fluorine (*14*) and the difficulties in making an artificial sea water were noted. Later it was found that after sea water was evaporated for easier transportation the fluorine would not redissolve. In a study of nutrition of rats, evaporated sea water was fed (*13*) and sea water was added to the drinking water. A study of the solubility of fluorapatite in saliva (*18*) led to the idea that fluorine is beneficial to the teeth. When fluorapatite was used as the sole source of calcium and phosphorus in rats' diet no evidence of fluorine poisoning was noted (*12*).

In a search for micromethods for fluorine analysis the assistance of A. G. Mulder (*16*) and W. D. Armstrong (*2*) was obtained. Since then Armstrong (*1*), Willard and Winter (*19*), Hoskins and Ferris (*8*), Churchill, Bridges, and Rowley (*3*), Jacob, Reynolds, and Marshall (*9*), McClure (*15*), Clifford (*4*), Dahle, Bonnar, and Wichmann (*6*), Reynolds and Hill (*17*), and others have contributed to the microanalysis of fluorine.

Notwithstanding the precise work on the fluorine content of teeth, bones, and phosphate rocks, the meager reports on dietary fluorine leave much to be desired. Cox, Matuschak, Dixon, Dodds, and Walker (*5*) state that "in agreement with Armstrong, Dahle, and McClure the value of analysis for fluorine on materials which must be ashed is questionable". The authors interpret this to mean that losses occur on ashing or that combustion products interfere with analysis. It therefore seems justifiable to publish the authors' more exacting technique, which has enabled them to find a diet (adequate for three generations of rats) so low in fluorine as to cause dental caries in 100 per cent of all rats. This diet has been made up in a number of batches which vary somewhat in fluorine content, but all contain about $^1/_3$ part per million of fluorine.

Whereas thorium tetrafluoride may be formed when thorium is the only cation in addition to hydrogen, in the presence of sodium ion it is possible to have $ThF_4.NaF$ which disturbs the stoichiometric titration of fluoride with thorium, and some failures are apparently caused by adding sodium hydroxide to distillates.

A spectrophotometric study was made of the thorium-alizarin-fluorine interaction, determining the pH with the glass electrode. The absorption curves (logarithm of reciprocal of transmittance) of alkaline alizarin (red) and of acid alizarin (yellow) are given in Figure 1. Since the Coleman spectrophotometer had a slit 300 Å. wide, the peaks of the absorption curves may not have been sharply determined.

The alizarin-thorium lake (dashed line, Figure 1) shows nearly the same absorption spectrum as the alizarin, but because the lake precipitates at pH greater than 4 except in extreme dilutions and low ionic strength, and the cell of the authors' Coleman spectrophotometer is only 19 mm. deep, the presence of a trace of the acid form of the alizarin could not be excluded. The peaks of the two absorption curves appear to be at the same wave length (5100 Å.). The difference in color is due to less absorption by the lake in the longer

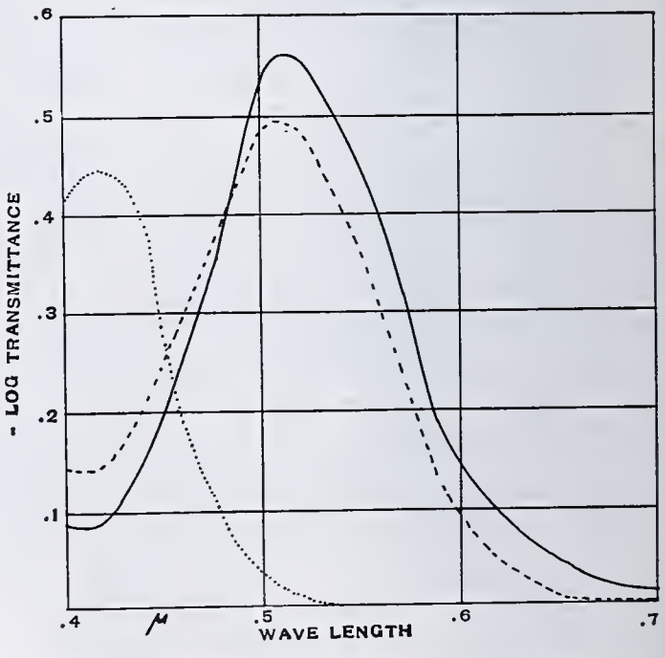


FIGURE 1. ABSORPTION CURVE OF ALKALINE AND ACID ALIZARIN AND ALIZARIN-THORIUM LAKE

1 cc. of sodium alizarin sulfonate in 25 cc. of 0.1 *N* hydrochloric acid (dotted line) and in 25 cc. of 0.01 *N* sodium hydroxide (solid line), and of thorium lake (dashed line)

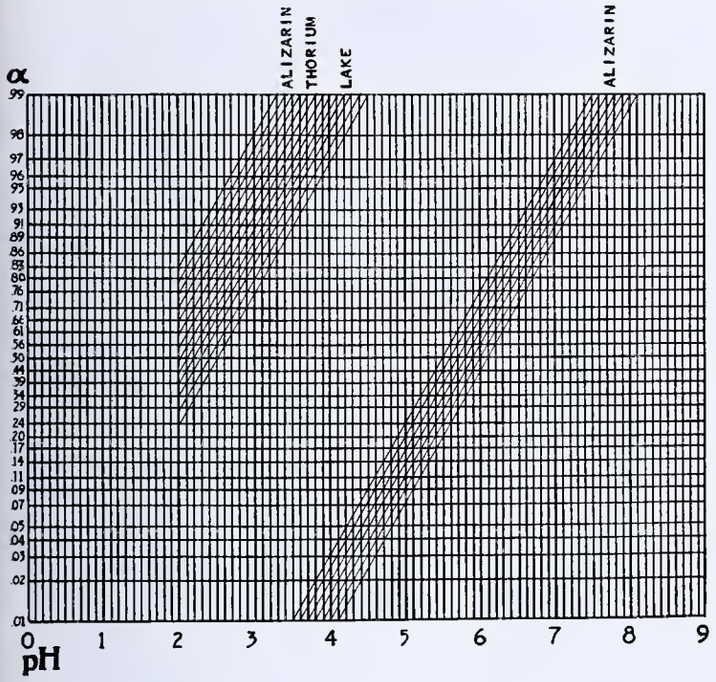


FIGURE 2. CHANGE IN DISSOCIATION OF ALIZARIN AND ALIZARIN-THORIUM LAKE WITH CHANGE IN pH

wave lengths. It therefore appears probable that the basis of the thorium procedure is the change in ionization constant of alizarin produced by thorium, and the following procedure is based on that hypothesis.

The results of pH-spectrometric measurements are plotted in Figure 2 on special graph paper (for sale at the Hahnemann Book Shop at 1 cent per sheet). Kolthoff (*10*) has shown that changes in ionic strength may change the logarithm of the reciprocal of the apparent ionization constant of alizarin as much as 0.06. The ionization, α, of alizarin with change in pH is represented as a broad band in Figure 2. The breadth of the band may be due to changes in ionic strength and room temperature. The point is that under ordinary conditions, between pH 3 and 3.5, alizarin is never more than 1 per cent ionized (red), whereas the thorium lake is more than 50 per cent in the red form. The thorium-alizarin lake is not so simple as the alizarin, and is shown as a guide to titration. The band is not continued to pH values less than 2 because the pH of distillates is never less than 2. The determinations of α were made at 5400 Å. at which the undissociated dye shows practically no absorption, whereas the ionized dye and thorium lake show nearly maximum absorption (see Figure 1).

From the data in Figure 2 it follows that the thorium solution should be standardized at the exact pH, ionic strength, and temperature at which the fluoride is titrated. The pH should not be greater than 3.5, and since reduction in the pH causes reduction in the red color and consequently in the sensitivity of the method, it is not wise to have the pH less than 3.

The acidity of the distillate is kept as low as possible by an efficient trap and the addition of silver sulfate or perchlorate to the distilling flask. The distillate is brought to pH 3 by addition of minute amounts of perchloric acid or sodium hydroxide and matched with a blank at the same pH.

Apparatus

Since open ashing leads to loss of chloride, and potassium chloride and fluoride have the same sublimation temperatures, there is danger of loss of fluoride on open ashing.

To avoid such losses, the closed combustion tube system shown in Figure 3 was adopted. This consists (from left to right) of a Pyrex absorber with three sintered Pyrex disks (from the Corning Glass Works, Corning, N. Y.), a platinum combustion tube, and an automatic stoker.

The furnace is made of two firebricks set with their broad faces parallel, 4 cm. apart, and covered with bright metal. They rest on a rectangular 3.785-liter (1-gallon) tin can laid on its narrow side with a slot 4 cm. wide cut lengthwise of the side turned uppermost. Convenient holes are cut in the front, back, and bottom of this can to operate three large Fisher burners. The can is blocked to prevent the tops of the burners from projecting more than about 1 mm. up between the bricks, when they will be overheated and strike back. The table top and rubber tubing are protected with high-temperature aluminum paint (from the Chemical Rubber Co., Cleveland, Ohio). The platinum combustion tube is 10 mm. in outside diameter at one end and 14 mm. in bore at the other. A 52-mesh platinum grid is pushed into the tube to prevent cinders from entering the absorber. The end of the absorber should be flared so that the small end of the combustion tube can be pushed into it about 1 cm.

A piece of 10-mm. stainless steel tubing about 30 cm. (1 foot) long [18–8 type 304 stainless steel tubing 0.788 cm. (0.315 inch) in inside diameter and 0.985 cm. (0.394 inch) in outside diameter, made by the Bishop & Co. Platinum Works, Malvern, Penna., which also makes the platinum parts] is used to introduce the sample into the wide end of the combustion tube. To make the connection, a platinum "stopper" is used, perforated with a 10-mm. hole for the stainless steel tube and a 3-mm. hole to admit oxygen. This stopper is cooled by a water jacket with 3-mm. inlet and outlet tubes.

A 0.625-cm. (0.25-inch) rod of stainless steel 35 cm. (14 inches) long is threaded with an adjustable 0.625-cm. (0.25-inch) die, 20 threads to the inch. The threads are cut in only halfway, leaving a "square" shoulder. A thimble-shaped cap of platinum 0.1-mm. thick is fitted loosely over one end of the rod.

Into one end of the stainless steel tube are driven 2.5 cm. (1 inch) of 0.625-cm. (0.25-inch) copper tubing 0.104-cm. (0.042-inch) wall (such as is used for the gas line in autos). The threaded rod is inserted through this end. A setscrew is inserted through the stainless steel and copper and into one of the threads in the rod. The other end of the rod is attached by means of a short piece of rubber tubing to the axle of a Telechron motor (B-2, 1 r. p. m., 60 cycles) that slides horizontally on a knitting needle.

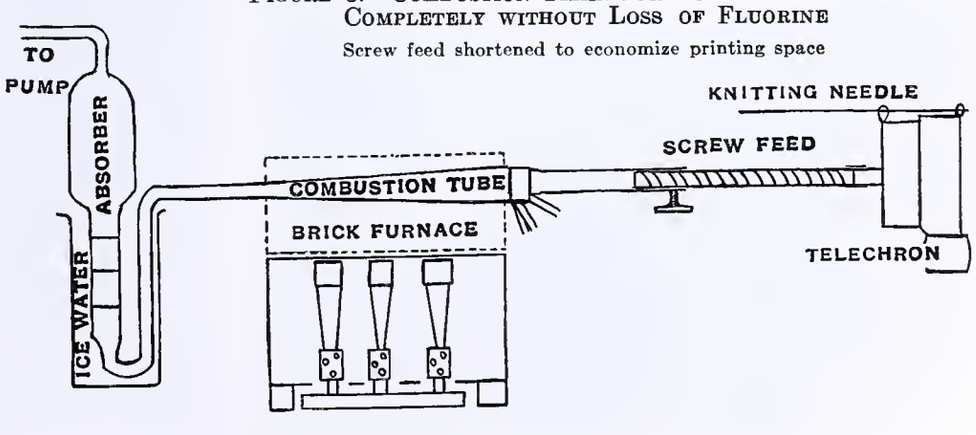
A Hoke micrometric control valve and Corning flowmeter are used to pass 200 cc. of oxygen per minute through the water-cooled platinum stopper.

The 50-cc. Pyrex microstill (made by the Precision Thermometer and Instrument Co., 1434 Brandywine St., Philadelphia, Penna.) is shown in Figure 4. The trap, which avoids the necessity of double distillation, is sufficiently close-fitting so that the water seal is not blown out during distillation. The filling tube has a Y at the top and the filling is done through the vertical arm. The lateral arm is used to pass in superheated steam at 150° C.

Steam is generated by boiling alkaline distilled water in a Pyrex flask with a Nichrome wire wrapped around its neck.

FIGURE 3. COMBUSTION TRAIN FOR BURNING FOOD SAMPLES COMPLETELY WITHOUT LOSS OF FLUORINE

Screw feed shortened to economize printing space



Two garden hose coupling clamps are attached to the two ends of the neck of the flask; 3.6 meters (12 feet) of 30-gage Nichrome wire and 3.6 meters (12 feet) of coarse sewing thread are attached to one clamp and wound together around the neck and the other ends are attached to the other clamp. A thick suspension of fireclay in water is poured over the winding and allowed to dry. Asbestos paper is wrapped around the fireclay and held with wire. The two clamps are connected with the two ends of a 110-volt electric circuit in series with a 200-watt lamp and switch. The flask is fitted with a 2-hole rubber stopper; in one hole is inserted a thermometer with its bulb in the neck of the flask and in the other a short piece of glass tubing to connect with the distilling flask. The temperature of 150° in the issuing steam is controlled by turning the switch on and off.

The vertical form of the distilling flask permits its being immersed in a Pyrex beaker filled with white mineral oil such as is used for constipation, and avoids the necessity of a thermometer inside the flask. The beaker also contains a thermometer and is heated by gas flame to 150° C.

The titrations are made in tall 100-cc. Nessler tubes with Lochte and Hoover (*11*) microburets (0.2 cc. graduated in 1000ths, from Williams, Brown and Earle, Philadelphia, Penna.). Titrations and pH determinations are made in a double-walled cellophane chamber about 210 cm. (7 feet) cube kept at a constant temperature of 25° C. by an electric thermostat, heater, and fan. The cellophane chamber is inside a steam-heated room. A small electric refrigerator unit is used to cool the chamber when the room is a few degrees too warm.

Reagents

Fluorine-Free Water. Distilled water is made alkaline with sodium hydroxide and redistilled in a Pyrex still.

Fluorine-Free Perchloric Acid. Ten cubic centimeters of Baker's analyzed 70 per cent perchloric are distilled with steam at 150° C. in the microstill and 100 cc. of distillate are tested for fluorine. If as much as 1 microgram of fluorine distills over, the residual acid is used as reagent; otherwise this purification is omitted.

Thorium Nitrate Tetrahydrate, 7.26 grams dissolved in fluorine-free water to make 1 liter. This is intended to correspond to 1000 micrograms of fluorine per cc. Some of this is diluted 10 times and some 100 times with fluorine-free water.

Sodium Fluoride (Baker's analyzed) is dried at 150° C. and cooled in a desiccator and 2.2104 grams are weighed and dissolved in fluorine-free water to make 1 liter. Some of this is diluted 10 times and some 100 times with fluorine-free water. These solutions are kept in paraffin-lined bottles, made by filling cylindrical glass-stoppered bottles one-fifth full of melted 60° paraffin and rolling them on the table until cooled. These solutions are labeled 1000, 100, and 10 micrograms of fluorine per cc. (A possible error of a very few per cent in the purity of the standard is within the limit of error in the determination of dietary fluorine.)

Powdered Quartz is heated with sulfuric acid and washed with fluorine-free water.

Titrations are made in tall 100-cc. Nessler tubes against a white table top with a 20-watt fluorescent lamp 15 cm. (6 inches) above it, shielded from the eyes of the operator. The table top must be of uniform whiteness, the bottoms of the Nessler tubes clean, and the eye trained by practice.

Procedure

Sufficient dry foodstuff to contain 1 microgram or more of fluorine is ground, weighed, and introduced into lengths of 0.938-cm. (0.375-inch) cello-dialyzer tubing (from the Fisher Scientific Co., Pittsburgh, Penna.). The casing is tied with thread at each end, the excess is cut off, it is somewhat flattened between two sheets of clean paper, and a knitting needle is pressed into one side. It is then wound with sewing cotton and the needle is withdrawn. The casing is dried in a steam-heated "ice maker" and introduced into the stainless steel tube. The platinum cap is placed on the end of the threaded rod and it is inserted as far as the end of the casing. The setscrew is tightened so that its point enters the thread of the rod.

The combustion train is assembled. The absorber is charged with 10 cc. of fluorine-free water and 0.2 gram of sodium hydroxide (and 50 mg. of sodium azide to destroy nitrous acid from ashing of the sample). The inlet tube is passed through a piece of aluminum foil (or kraft paper coated on each side with aluminum foil) to reflect the furnace heat, and immersed in ice water. The outlet tube is connected to an aspirator (with vacuum storage tank if water pressure varies) through a Hoke micrometric control valve. The inlet tube is connected with the platinum combustion tube in the furnace, the platinum stopper is inserted, the cooling water and oxygen are connected, and the threaded rod is connected by means of a short piece of rubber tubing with the Telechron motor sliding on a knitting needle. The burners are lighted and a piece of asbestos board is used to cover the middle portion of the furnace top. About 200 cc. of oxygen per minute are admitted and suction is applied to maintain atmospheric pressure inside the combustion tube, counting the bubbles in the absorber or attaching a delicate manometer by means of a T-tube to the oxygen inlet. There should be no noticeable back pressure in the passage of the oxygen through the 3-mm. opening. When the combustion tube reaches bright luminosity the Telechron motor is started. In case the sample fills more than one length of casing, these lengths may be fed into the stoker one at a time by loosening the setscrew and removing the threaded rod.

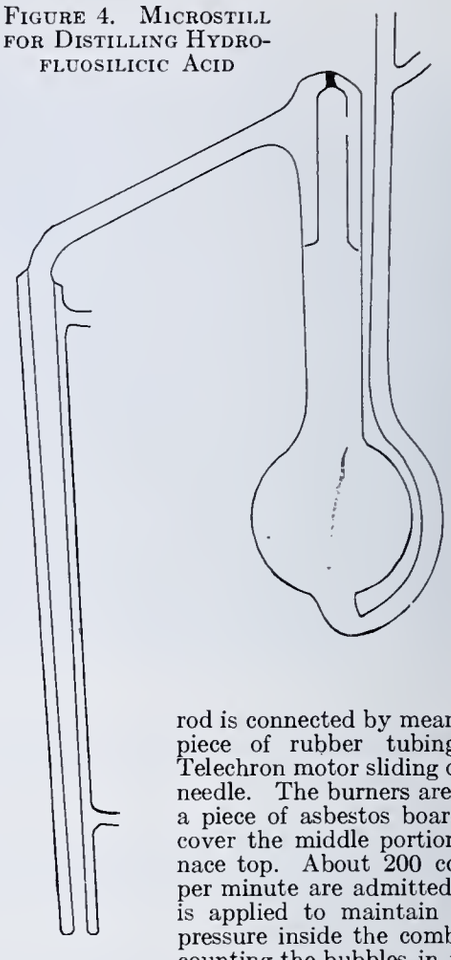


FIGURE 4. MICROSTILL FOR DISTILLING HYDROFLUOSILICIC ACID

When the casing is all fed into the combustion tube the platinum cap is allowed to drop off the rod, and the rod is withdrawn after loosening the setscrew. The heating is continued 5 minutes to burn the tar off the cap. The cooling water is reduced until it boils and the heating continued 5 minutes more. The rubber tubes are disconnected from the platinum stopper and it is allowed to heat for 5 minutes while air is drawn through to burn the tar from its inner face. The gas and suction are turned off and the contents of the absorber and the ash and washings of the platinum tube with 3 cc. of 0.1 *N* perchloric acid are transferred to the microstill with 0.1 gram of powdered quartz and enough silver sulfate or perchlorate to precipitate all the chloride.

Heat is applied to the oil bath to heat it to 150° C., while compressed air is led slowly through the filling tube to distill off most of the water. If the sample is reduced to 2 cc. before the oil bath reaches 150°, the still is raised and reinserted when the temperature is correct.

The condensing water must be running and a 100-cc. Nessler tube used as a receiver when 10 cc. of perchloric acid are introduced through the filling tube, and the orifice closed after the superheated steam is introduced from the superheater (previously heated to 150°).

The distillation with steam is continued until 100 cc. of distillate are collected. A blank distillation is also made. After mixing by pouring in a beaker the pH is determined with a glass electrode and the portion used to determine pH is returned to the Nessler tube. If the pH is greater than 3, the calculated amount of perchloric acid is added to the unknown and to the blank to bring them to 3. The pH of these solutions is then verified or adjusted to be the same with the glass electrode.

If the pH is less than 3, sodium hydroxide is added to the unknown and blank in a similar manner. (If the pH is only slightly less than 3 it may be diluted with fluorine-free water until the pH is 3, and a 100-cc. aliquot taken for analysis.) The rate of distillation may be regulated so that the pH is about 3.

The unknown and blank should be colorless; with each is mixed 0.1 cc. of 0.04 per cent aqueous sodium alizarin sulfonate and they are matched for color. The microburets are filled and

their tips wiped with San Fay tissue. Thorium solution corresponding to 1 microgram of fluorine is run into the blank, which should have a perceptibly pinker color than the unknown. Thorium solution is run into the unknown from a microburet until it matches the blank. The contents are throughly mixed by pouring in and out of a beaker; about 20 seconds are required to develop full color. The titer is recorded and an equal total amount of thorium added to the blank (remembering that some was added at the start), which should be perceptibly pinker. If no difference appears the fluorine in the unknown must be recorded as zero, but this has not occurred in the authors' work except in distillates from purified reagents.

If the blank is pinker than the unknown, fluoride solution is added until the colors match again, when the fluorine content of the unknown is equal to that added to the blank. A number of unknowns may be titrated, provided they have the same pH as the blank. After thorium equivalent to 1 microgram of fluorine is added to the blank, each unknown is titrated with thorium to match it in color. Beginning with the unknown to which the smallest amount of thorium was added, the blank is back-titrated to each unknown in turn.

Table I. Recovery of Fluorine Added to Rats' Bones and Teeth

	F	Recovery
	Micrograms	%
Femur, 117.7 mg.	3.0	...
Femur, 117.7 mg. + 10γ F	13.4	104
Teeth, 162.6 mg.	3.5	...
Teeth, 162.6 mg. + 10γ F	14.0	105

Results

A comparison of the authors' microstill with those devoid of traps shows that negligible amounts of perchloric acid pass their trap on distilling with steam at 150° C. With slow distillation the pH of the distillate was 4.6 (after standing), whereas that of water redistilled in Pyrex from sodium hydroxide without trap was 5.5, but after standing (and absorbing carbon dioxide from the air) was 5.05. This indicates that the normality of the acid in the distillate was about 0.00014. In addition to the 0.00014 *N* perchloric, the acids that might come over in analyses are halogen and nitrogen acids and sulfuric acid. The former (except the fluorine) are held back by silver. The substitution of silver sulfate for silver perchlorate did not noticeably increase the acidity of the distillate, but increasing the rate of distillation does increase the acidity.

The recovery of fluoride added to rats' bones and teeth is shown in Table I.

Table II. Fluorine in Teeth and Bones of Rats on Diet Analyzed in Table III

Rats	Fluorine Found Enamel	Fluorine Found Dentine	Rats	Bone	Fluorine Found
	%	%			%
1 and 2	0.004	0.010	4	Left femur	0.007
3 and 4	0.010	0.010	4	Right femur	0.008
5 and 6	0.004	0.007	5	Right femur	0.010
			6	Right femur	0.010
			7	Right femur	0.010
			8	Right femur	0.004

Table III. Fluorine in Rats' Diet and Recovery of Added Fluorine

(Diet, 5 grams)

Fluorine Added	Fluorine Found	Recovery
Micrograms		%
0	1.5	...
10	12	104
0	1.5	...
10	11.3	98
0	1.9	...
5	7	101
0	1.8	...
2.5	4.5	105
0	1.8	...
1	3	107

The only analyses of fluorine in rats' dentine and tooth enamel found in the literature were made by Armstrong (7); the fluorine in the diets was unknown, although one diet was known to contain more fluorine and produced "mottled" teeth. Armstrong found (average) 0.0082 per cent of fluorine in "normal" enamel and 0.0109 per cent in the dentine. He found (average) 0.196 per cent in "mottled" enamel, and 0.257 per cent in the dentine.

The authors have analyzed the fluorine in the bones and teeth of rats receiving from weaning about 0.33 microgram of fluorine per gram of diet as shown in Tables II and III.

Summary

In determining fluorine in the presence of large amounts of organic matter, a closed system of ashing prevents the loss of fluorine by volatilization.

An improved still allows separation of fluosilicic acid in a single distillation. The perchloric acid carried over may be 0.00015 *N* and does not interfere with the titration.

The interference of sodium ion may be due to the formation of $ThF_4.NaF$, precipitation of the lake, and effect of change in ionic strength on alizarin.

At pH greater than 3.5 there may be error due to free alizarin in red form. At pH less than 3.5 the thorium lake does not show its full color; therefore the thorium must be standardized with fluoride at exactly the same pH at which the titration is made. The back-titration on a blank distillate after adjusting pH with the glass electrode allows this to be accomplished, as well as correcting for fluorine in reagents.

From 2.5 to 10 micrograms of fluorine added to the sample may be recovered with an error of 5 per cent. The greater percentage inaccuracy with smaller quantities may be due to fluorine in the reagents and to the uncertainty of the end point. It appears that the color of the thorium lake is not entirely suppressed by adding the equivalent of fluoride. The method is considered valuable in determining 1 microgram of fluorine in foodstuffs (error, 7 per cent).

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MODERN LABORATORIES

Laboratories of the Best Foods, Inc.

H. W. VAHLTEICH, Best Foods, Inc., Bayonne, N. J.

THE new research and development laboratory of the Best Foods, Inc., is a $2^1/_2$-story rectangular brick building about 50 by 80 feet. It was designed by Lockwood Greene Engineers, Inc., in cooperation with the laboratory staff, which is also responsible for the special features and equipment layouts. Its purpose was to consolidate previously scattered laboratory operations, to supplant outworn and outmoded facilities with up-to-date equipment, and to provide room for expansion. Extensions on one or more of three sides may be made with a minimum of inconvenience.

In a number of practical aspects the design is unique for a rectangular building—for example, the main entrance is at one corner. This corner is built in the form of a semicircular tower, a feature which permits the following practical applications: Four stories are provided in the tower; a low basement level for a darkroom, a ground-level entrance foyer, and offices on each of the two upper floors; a pleasant circular effect in the offices and foyer as a relief from the usual straight-sided rooms; shorter halls inside the building with resulting economy of working space; and a complementary inside circular rotunda, facing which are a library, a model kitchen, and an experimental bakery. The object is to facilitate tours of consumer visitors who can, without being wearied by complicated laboratory equipment, gain a general impression of the organization's care in providing for technical control of its products. Large curved plate-glass windows are set in the glass-block walls of the rotunda lobby to permit an unobstructed view of technicians at work.

Another unique design feature is the safety balcony, extending along the north and west sides of the building, which provides means of egress from all rooms.

Headroom on the top floor is 12 feet, on the first floor 13 feet, and in the basement 14 feet; however, a 20 × 20 foot bay in one corner of the basement is 4 feet below floor level, providing 18 feet of headroom for tall pilot-plant equipment, and in one corner of this bay steel reinforcing in the concrete is omitted from a 5 × 5 foot ceiling area to permit easy removal should an apparatus column up to 31 feet be desired. Tile walls are used throughout all laboratories to avoid expensive repainting, and plywood forms for all laboratory ceilings gave a ceiling sufficiently smooth to paint on directly without the use of plaster. Hauserman steel partitions carrying glass above 3 feet are used to section off working space and simultaneously permit easy inspection of working spaces from a distance. Handrails and doors are of similar metal construction.

Floors are concrete with a 0.75-inch dressing of steel-gray cement, with the exception of terrazzo in the first-floor passageways, kitchen, bakery, and entrance lobby.

Lighting, which has received special attention, in all rooms is of the high-intensity indirect type, a circular hanging fixture with plastic shields and reflecting globes having been used. Glass brick has been used freely for both interior and exterior walls, and this, together with large windows, affords well-lighted rooms and laboratories. Throughout, steel sash and casement-type windows have been used, while wire glass instead of plate glass in many instances provides an additional safety measure. Offices, library, and corridors have acoustical ceilings. Under the roof is a 2-inch layer of cork, which, together with the high headroom, minimizes discomfort due to heat in summer.

To prevent water from entering electrical outlets when rooms are flushed in cleaning, all sockets have been placed 2 feet above the floor. The electrical system is 4-wire polarized, making available at each outlet 120- and 208-volt, 1- and 3-phase current. Every room has a master panel which controls individual outlets on benches and work tables. Laboratory benches are also provided with 110-volt direct current.

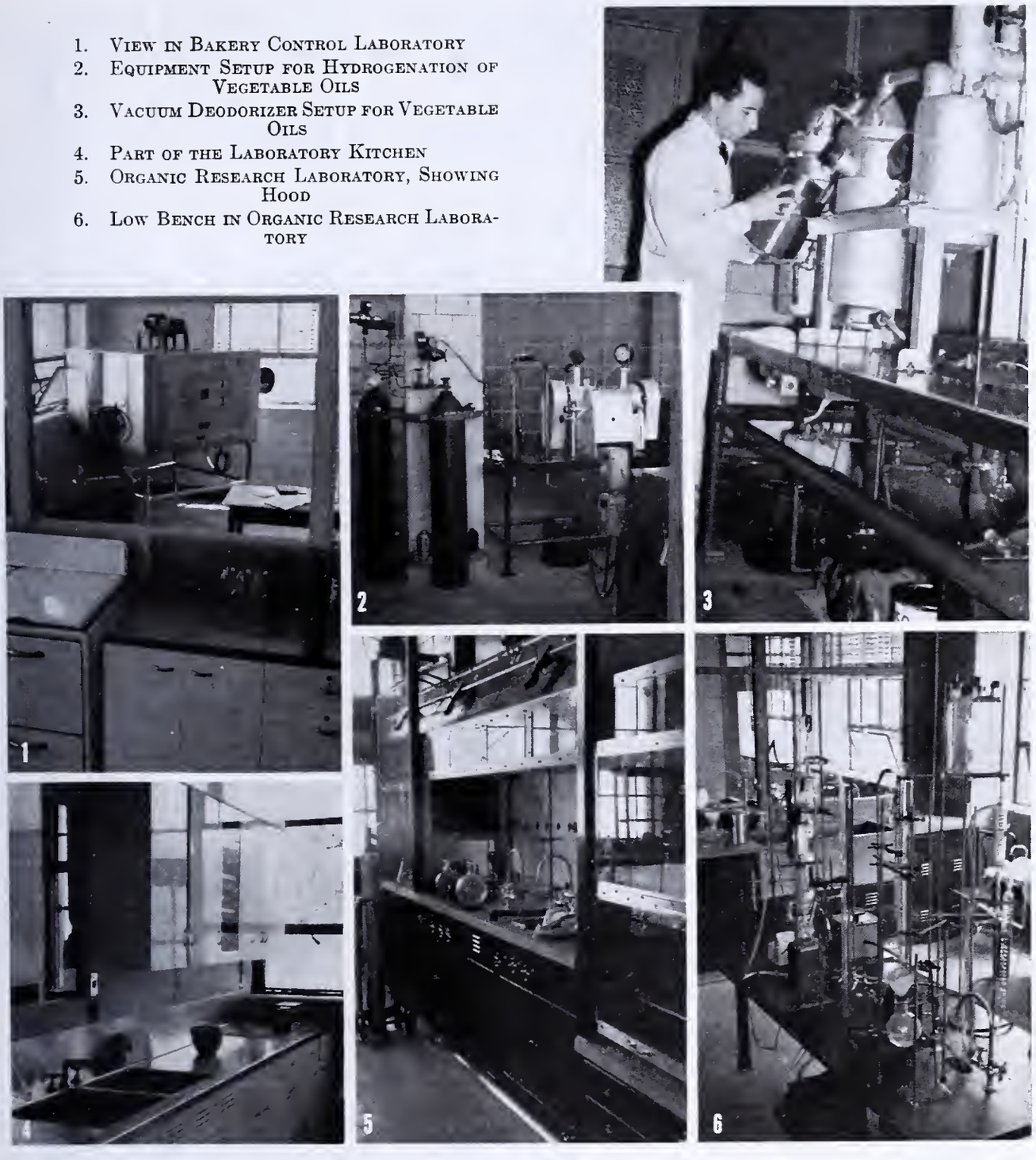
Ceilings purposely have been made high to aid ventilation, and to remove piping from the line of vision. Pipes in the walls are readily accessible, being housed behind removable metal partitions.

Instead of a sprinkler system, the A. D. T. system of fire detection has been installed. Primarily, this method of detection relies upon the pressure increase of air, within a fine copper tube, as the temperature within a room increases. Sudden increases will cause an alarm to sound, but slower changes due to normal conditions have no effect, as provision is made for the escape of gradually heated air.

The laboratory furniture and equipment, like the building, were designed to meet a variety of special requirements. The furniture is steel of the double-wall type, lead-coated and finished in laboratory green. Alberene stone tops are used throughout. Drawers are on roller bearings and the ends of all benches include recessed sinks and waste cans. Interiors of all furniture have been lacquered in white to facilitate finding things, a feature found practical from previous trials.

In providing for about 70 lineal feet of hood space it was also necessary to provide for properly distributing and heating the incoming air when the hoods are in operation. This has been done by means of Trane Torridor units, synchronized to operate automatically when the hood fans are turned on, and thermostatically controlled to provide uniform room temperature in cool weather. Both features are achieved by means of a Johnson system of control. The hoods have the usual dual control system, providing equal ease of operation from within and from the front of the hood when the glass doors are down. The large hoods are in three sections separated

1. View in Bakery Control Laboratory
2. Equipment Setup for Hydrogenation of Vegetable Oils
3. Vacuum Deodorizer Setup for Vegetable Oils
4. Part of the Laboratory Kitchen
5. Organic Research Laboratory, Showing Hood
6. Low Bench in Organic Research Laboratory



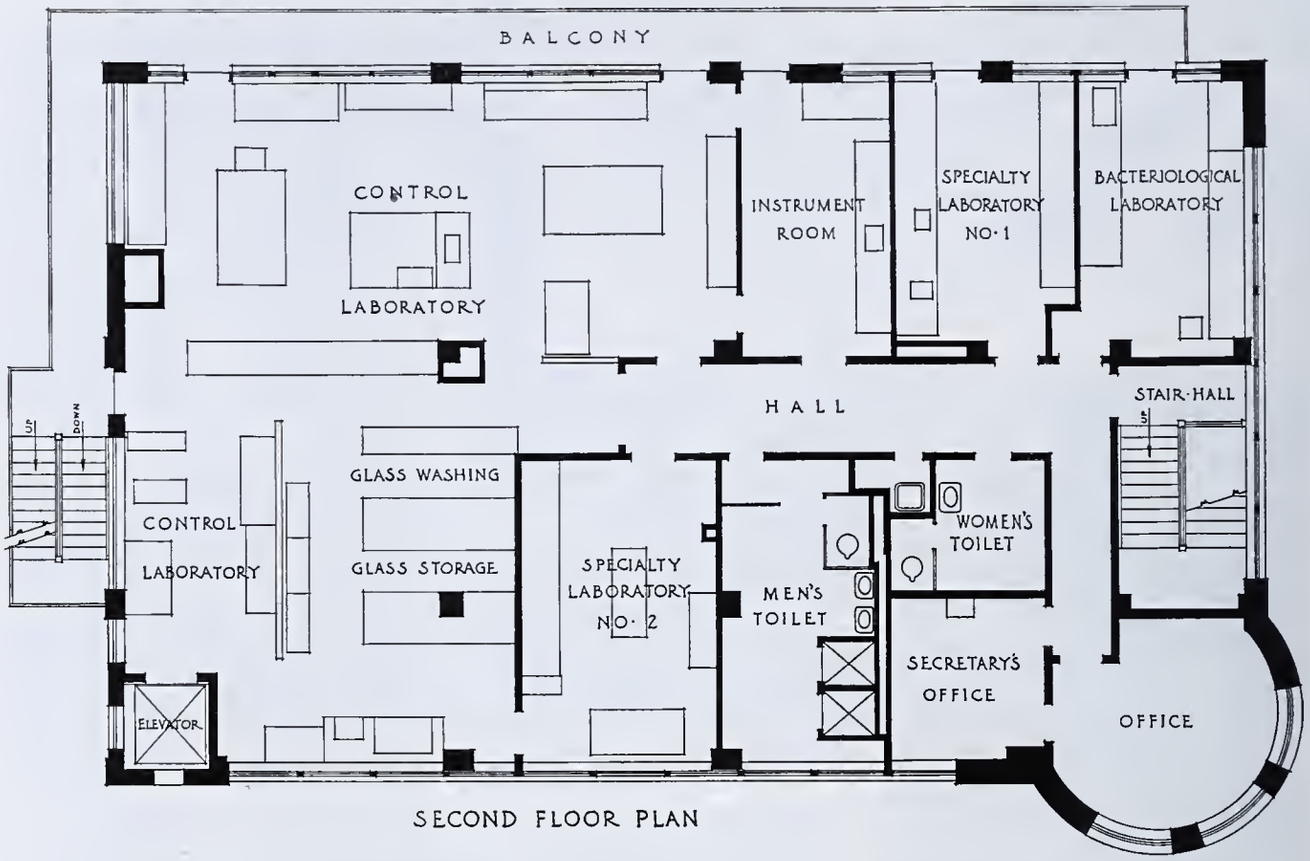
by transoms, and may be operated as one hood or in three sections as desired. One end section of each hood is provided with two flush hot plates, one steam and the other electrically heated. A large hood in the organic research laboratory is provided with one low section to facilitate the handling of tall apparatus. All hoods are provided with steam-heated cabinets and Transite-lined cabinets for corrosive liquid containers. All hood ducts lead to the roof, are of Transite lined with pitch, and the Transite sections are removable.

All laboratories are provided with at least one safety shower, as well as with modern equipment for fighting small fires. One laboratory with hood space has been set aside for analytical work requiring inflammable solvents. Here there are no electrical switches and no gas whatever is provided; all heating must be done with steam. All electrical equipment in this room is of the explosion-proof type (lights, unit heater and hood motors, mercoid control, etc.). The usual doorknob is replaced by a special device as an additional reminder that smoking is not permissible.

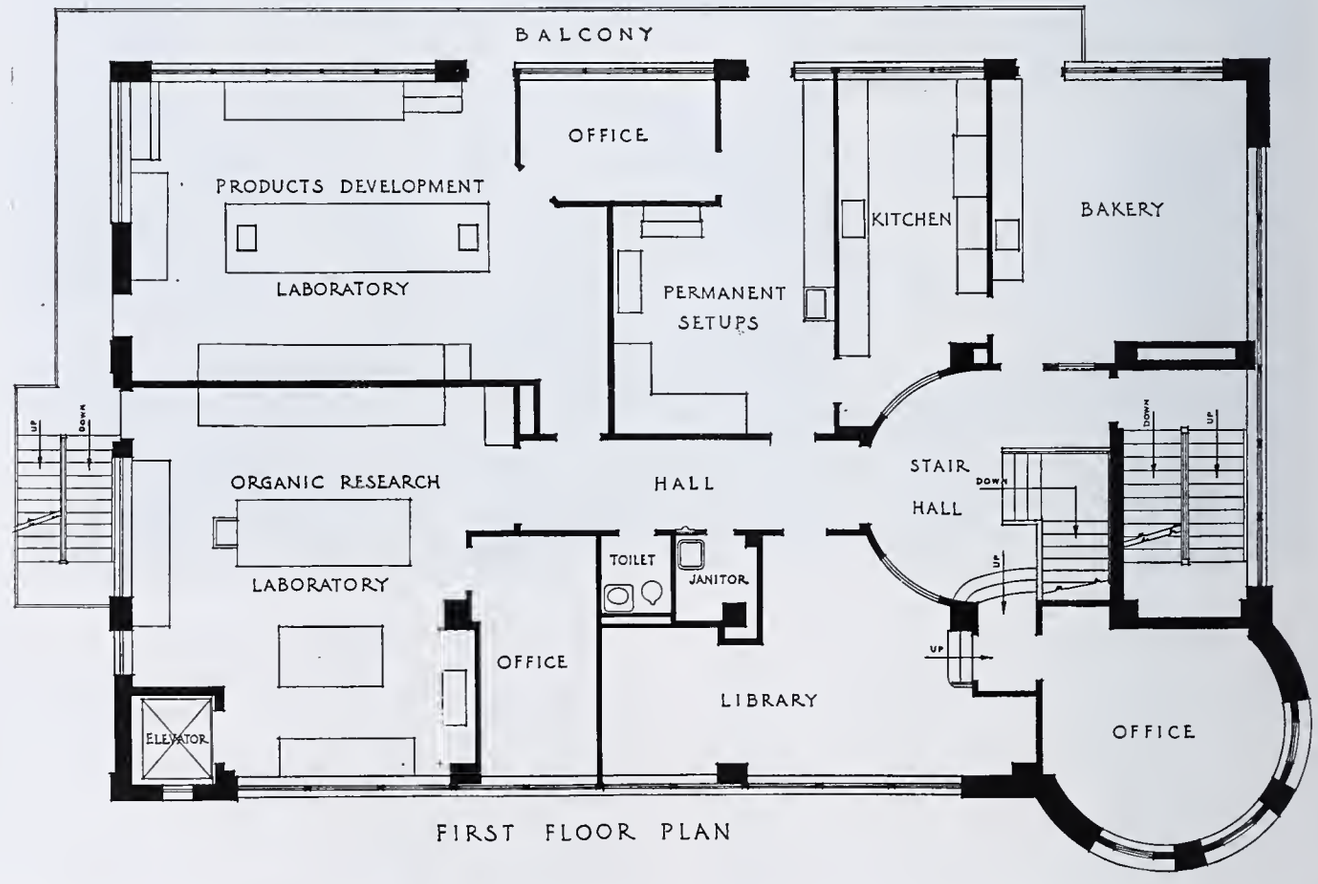
Another special laboratory has been set aside for unusually dusty or dirty work. The basement has been largely reserved for pilot-plant work. It also houses the photographic darkroom with a convenient light lock for passing in and out without disturbing work in progress. Four constant-temperature rooms, two cold and two warm, are also provided in the basement, as well as a large storeroom, filing space, and housing for the freight elevator and compressed air machinery. Vacuum is widely used throughout the building, but is supplied by individual electrically driven pumps.

There are also an experimental bakery, a model home kitchen, a permanent "setups" room for small-scale hydrogenation equipment, a small-scale edible oil deodorization system and oil-refining equipment, a bacteriological laboratory, etc.

The apparatus-washing facilities are largely concentrated adjacent to the analytical laboratory on the second floor, where they are most needed, but special washing facilities are also provided in the organic research laboratory. Dis-



SECOND FLOOR PLAN



FIRST FLOOR PLAN

tilled water is produced adjacent to the analytical laboratory and piped to the first floor. One end of the analytical laboratory is walled off to provide a room for housing instruments. Here the chemist may work in relative quiet and is also provided with a means for darkening the room when using such instruments as the polariscope.

Services to laboratory benches (including fume hoods) include hot and cold water, live steam, gas, electricity, and air. Stainless-steel steam baths are provided in several locations on the benches and hoods. A Schwartz sectional system in the analytical laboratory houses hundreds of samples of all sizes and shapes out of sight and yet readily available. The organic research laboratory is provided with a special low bench of modern design. The library, one side of which forms the curved glass-block wall of the rotunda, has bookcases built by a laboratory furniture company like conventional laboratory glassware cabinets, but grained in a walnut finish and provided with plate-glass doors having narrow stainless-steel trim. A special telephone system is provided to ease the service load on the switchboard.

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Determination of the Heat of Combustion of Gasolines

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DETERMINATION of the heat of combustion of gasolines is becoming increasingly important and is included in certain aviation gasoline specifications. The application of this test to gasolines must be accompanied by a more closely controlled procedure than that ordinarily employed by petroleum testing laboratories for heavier fuels, in order to obviate losses of the more volatile components of the gasolines, such as butanes and pentanes, which have the highest heating values.

Considerable work has been published on the art of calorimetry since Andrews (*1*) first used the bomb calorimeter in 1848. A comprehensive bibliography on this subject is furnished by Kharasch (*14*). The apparatus, technique, and procedure of modern calorimetry have been described at considerable length by Dickinson (*4*), Jessup and Green (*10*), Richards and Gucker (*20*), Rossini (*23*), and White (*31*).

The work on the heat of combustion of hydrocarbons has been done mainly on pure compounds in order to study the relation between energy, structure, number of carbon atoms, and the influence of organic groups. For this purpose the apparatus and technique have been developed to such a high degree of precision and accuracy that, according to Rossini (*22*), measurements of quantities of energy can be made with uncertainties as low as 0.01 to 0.02 per cent. To attain this degree of accuracy considerable time, apparatus, and a precise technique are necessary. However, in the practical application of this test to gasolines, such precision is not warranted.

A procedure has been developed whereby six to eight determinations per day can be made on low-boiling (aviation) gasolines, with an accuracy of ±0.2 per cent.

Apparatus and Materials

A calorimeter of the adiabatic type was employed. This consisted of a double-valve oxygen bomb, a calorimeter bucket with stirrer, and a water-jacketed case equipped with a stirrer and connections to hot- and cold-water lines for maintaining adiabatic conditions.

A Beckmann thermometer calibrated by the National Bureau of Standards was used to measure the temperature rise. The jacket temperature was controlled with respect to the calorimeter bucket temperature by means of two 3-junction thermocouples and a galvanometer as shown in Figure 1. Slight differences in temperature between the bucket and the jacket are clearly shown by the galvanometer and thus a sensitive balance can be maintained.

The oxygen used should be at least 99.8 per cent pure and free from any combustible material. Commercial oxygen made by the liquid air process frequently contains as much as 0.3 per cent of hydrogen, possibly from contamination, and should be passed over copper oxide at 600° to 700° C. before being used for calorimetric work. Keffler (*11*, *12*) has shown that even electrolytic oxygen may contain considerable impurities. A number of cylinders of commercial oxygen made by the liquid air process were analyzed and found to contain from 0.01 to 0.3 per cent of hydrogen. Oxygen containing no carbonaceous material and an amount of hydrogen not exceeding 0.01 per cent was considered to be satisfactory without pretreatment.

Ordinary pharmaceutical capsules (No. 00) were employed. The ignition wire was pure iron and of No. 34 B. & S. gage. Combustion cups used were made both of platinum and illium. Benzoic acid used in calibrations was obtained from the National Bureau of Standards.

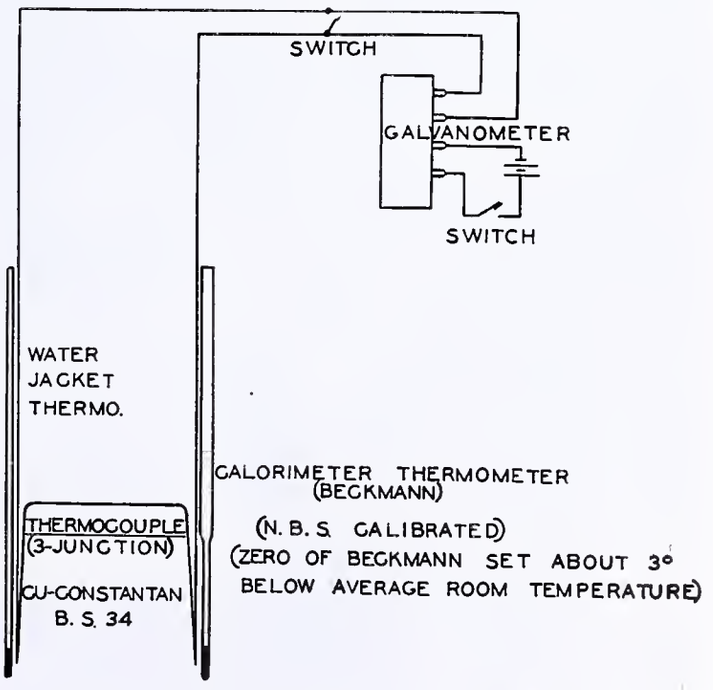

FIGURE 1. TEMPERATURE-CONTROL ARRANGEMENT

Procedure and Discussion

The procedure is standard in most respects, and is generally described in the literature. Calibrations were made using benzoic acid, the heating value of which was based on standards of e. m. f. and resistance maintained by the National Bureau of Standards. The heat units employed in the calculations were the same as those used by Rossini (*22*) which are:

1 calorie = 4.1833 international joules = 4.1850 absolute joules
1 absolute joule = 9.480×10^{-4} mean B. t. u.
1 calorie per gram = 1.7994 B. t. u. per pound

Sampling. A considerable saving in time is accomplished by basing calculations on a direct weighing of the sample rather than on a weight of sample burned as calculated from an analysis of the products of combustion. Jessup (*9*) has indicated that calculations on either basis are in close agreement when the sample is obviously completely burned.

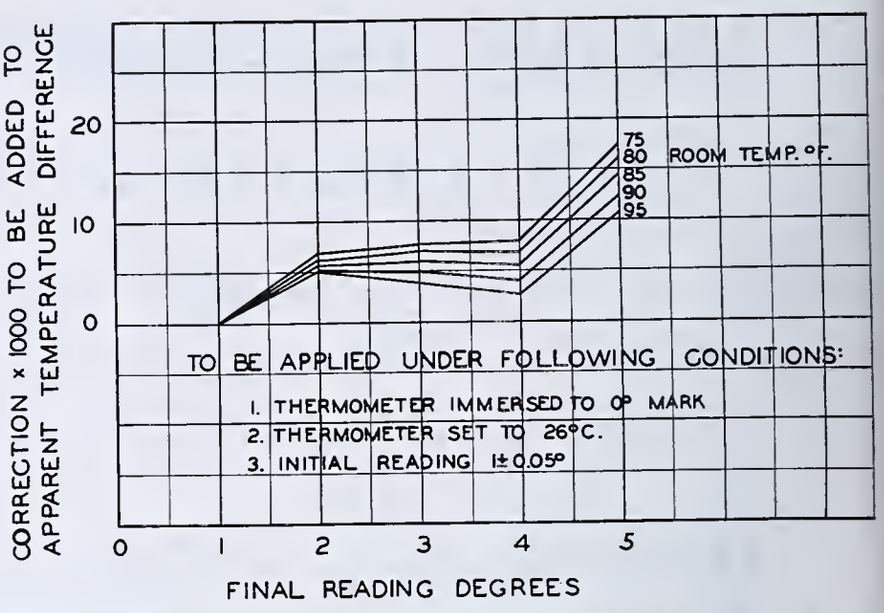

Figure 3. Example Correction Chart for Beckmann Thermometer

Thin-walled glass bulbs, flattened on two sides to withstand pressure changes, have been used by Richards and Barry (*19*), Jessup (*8*, *9*), and others. The advantages of the glass bulbs are that no corrections are necessary for the heat of combustion of the sample container, and that no absorption of moisture from the atmosphere is encountered. For complete combustion of liquid hydrocarbon samples enclosed in a container, such as a glass bulb or gelatin capsule, it is necessary that the container be completely filled with the liquid. This is done to prevent explosions within the oxygen bomb, upon ignition, with consequent scattering of the sample beyond the combustion zone, resulting in incomplete combustion. However, for gasoline samples containing considerable amounts of very volatile hydrocarbons, the use of glass bulbs is not recommended by the authors since, to fill these bulbs completely, it is necessary to heat and cool alternately.

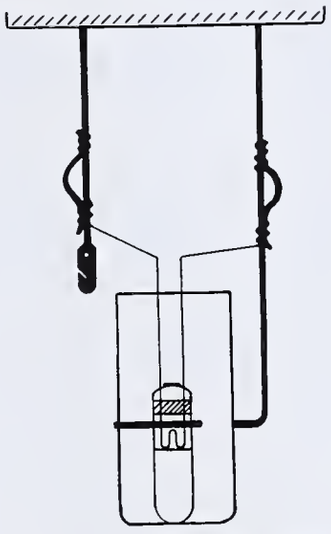

Figure 2. Ignition System

Accurate weights of representative samples of gasolines have been obtained by using gelatin capsules and employing a special technique for filling and sealing. When the capsules are first removed from their sealed container, a heat of combustion determination is immediately made on a composite of several capsules selected in a representative manner in order to establish a correction factor for the capsules. At the same time each of the remaining capsules is weighed, the weight being recorded on a slip of paper inserted within the capsule. This procedure eliminates any error in the calculated heat value of the capsule due to change in weight from subsequent moisture absorption.

The capsule is sealed by lightly wetting the perimeter of the smaller half with water and inserting it into the other half. This forms a completely sealed capsule which is reweighed before filling with the sample. The gasoline sample, cooled in an ice bath, is drawn into a hypodermic syringe and is then injected into the sealed capsule. The air is allowed to escape from the capsule through a small pinhole near the hole made by the hypodermic needle. When the capsule is completely filled the two holes are preferably sealed with a small drop of collodion or, when sealing samples of high volatility, by applying a small gelatin patch which has been previously weighed with the capsule and is wetted with water for sealing. The weight of this water (2 to 3 mg.) is estimated from a number of comparative weighings. If collodion is used, and is properly applied, its weight is negligible.

Where atmospheric conditions of high temperature and humidity are encountered, it is recommended that all sample handling be carried out in a room held at a constant low temperature and low humidity, so that when cold hydrocarbon samples are injected into the capsules no moisture condensation onto the capsules is encountered.

Sample Ignition. The capsule, completely filled with the sample, is suspended by means of the iron ignition wire near the bottom of an illium, stainless steel, or preferably a platinum cup, which is approximately 3.75 cm. (1.5 inches) deep, as shown in Figure 2. Although good combustion can be obtained in a shallow cup, the deep cup gives complete combustion more consistently for samples sealed in gelatin capsules. Various methods have been described by Richards and collaborators (*19*, *21*) to ensure complete combustion of a hydrocarbon sample. However, it has been shown by some investigators (*8*) that complete combustion can be obtained without using any foreign materials as combustion aids. In the work on gasolines it has been found that unburned hydrocarbons and carbon monoxide exist in negligible quantities whenever the sample burns with no visual carbon formation.

Jessup (*8*) has recommended using 30 atmospheres of oxygen pressure for complete combustion of hydrocarbons, while Richards and Jesse (*21*) claimed that with 35 atmospheres an explosion took place and with 20 atmospheres no carbonization occurred. In the experimental work on gasolines various oxygen pressures ranging from 15 to 40 atmospheres were used. It was found that the use of a pressure of 30 atmospheres resulted in the most consistent complete combustions.

Measurements. To attain the desired accuracy for gasoline work it is necessary to establish the following tolerances on the measurements involved:

Weights of sample and capsule	±0.1 mg.
Weight of calorimeter water	±0.1 gram
Thermometer (bucket) reading	±0.001° C.

Further, it is recommended that a sensitive galvanometer be used on the thermocouple circuit shown in Figure 1, so that

the analyst can control the jacket temperature to within 0.1° C. of the bucket temperature during the temperature rise following ignition of the sample. When obtaining the water equivalent of the calorimeter, by burning standardized benzoic acid, the amount of sample should be such that the temperature rise obtained will closely approximate the temperature rise that results subsequently when analyzing the gasoline samples.

Banse and Parks (*2*) report satisfactory results with the use of a Beckmann thermometer, while other investigators (*8*) employ platinum resistance thermometers for more accurate measurements. In the case of a calibrated Beckmann thermometer much time can be saved by the use of a chart giving the total correction to be added to the nominal temperature difference observed. This total correction, as illustrated in Figure 3, includes the scale, emergent stem, and setting factor corrections as calculated on the basis of a Bureau of Standards certificate received with the thermometer. In the construction of the chart the total corrections to be applied to the apparent difference between the starting and final temperatures are calculated for final readings of 2°, 3°, 4°, and 5° at each of several room temperatures covering the range encountered. The points are joined with straight lines, since an appreciable part of the total correction will be due to errors in the scale graduations which are difficult to estimate at points not calibrated.

Calculations. Corrections to the total observed heat of combustion values are made for the fuse wire consumed and for acids formed by the oxidation of nitrogen and sulfur. The latter corrections are relatively small for gasoline work, amounting to only 3 to 5 B. t. u. per pound for samples meeting customary specifications for sulfur. The methods to be applied in precision calorimetry for correcting the data to standard states are described in publications of the National Bureau of Standards, particularly by Washburn (*29*).

The net, or lower, heat of combustion values are the ones usually written into gasoline specifications. These are obtained by correcting the gross values, as determined, for the heat of vaporization of water formed. This is most satisfactorily accomplished by actually determining the weight per cent of hydrogen in the gasoline by means of a combustion analysis.

Table I. Reproducibility of Results

No.	Values	Maximum Deviation from Average	Average Value
	%	%	*B. t. u./lb. gross*
25	100	0.26	20,664
24	96	0.25	20,666
23	92	0.22	20,669
22	88	0.19	20,670
21	84	0.17	20,673
16	64	0.10	20,671

Table II. Characteristics of Control Sample

	n-Heptane	Literature Values
Boiling point, ° C.	98.4	98.4
Freezing point, ° C.	−90.68	−90.6
Refractive index, n_D^{20}	1.38769	1.38777
Specific gravity, d_4^{20}	0.6836	0.6837

Results. Reproducibility of results is exemplified by 25 determinations made on a sample of *n*-heptane, as shown in Table I.

The *n*-heptane used had the characteristics shown in Table II.

The values obtained by this method may be compared with those of the National Bureau of Standards, which reported values of 20,731 B. t. u. per pound for a different sample of *n*-heptane from the same source and 20,714 B. t. u. per pound for pure *n*-heptane (*24*). These data indicate that the errors of the method used result in low values. The authors recommend, therefore, that the procedure be followed closely with the suggestions offered in order to keep the results within 0.2 to 0.3 per cent of the true values.

Estimation of Heats of Combustion

For laboratories that are not equipped to make this test, or for rapid estimations of heats of combustion, a correlation has been derived to yield reasonable values from other data which may be more readily obtainable. In Figure 4 a linear

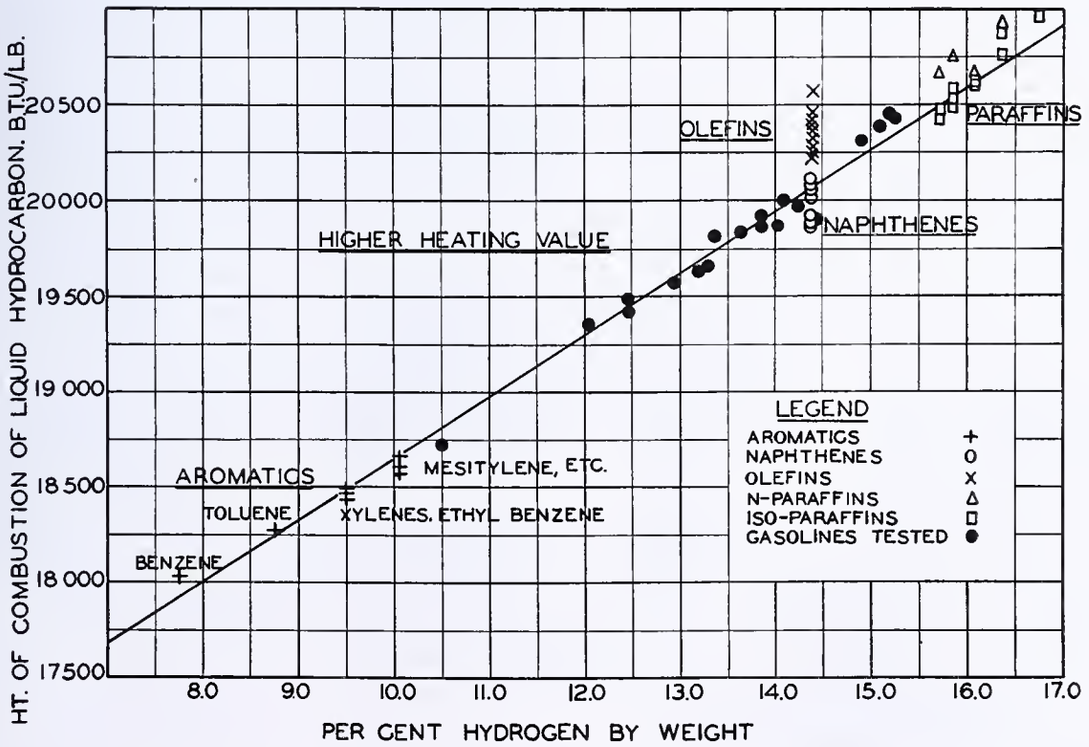

Figure 4. Correlation of Heats of Combustion with Per Cent Hydrogen

function is shown to relate the heats of combustion with hydrogen content. This correlation has been derived from the literature values for the heats of combustion of pure hydrocarbons boiling in the gasoline range, as given in Table III. The paraffin and aromatic hydrocarbons line up very well, but the naphthenes and olefins may diverge considerably. However, for gasolines of usual composition this curve may be applied. In Table IV there is tabulated a comparison of actual heats of combustion on gasolines with values estimated from the correlation in Figure 4.

The hydrogen content of gasolines is not generally determined by routine petroleum testing laboratories. Thus, in order to determine net heating values it is necessary to employ some means for obtaining reasonable approximations of the hydrogen content. Sweeney and Voorhies (*27*) present a correlation of hydrogen content with the average boiling point and gravity. The characterization factor, as given by Watson, Nelson, and Murphy (*30*), may also be employed to derive an approximate hydrogen content from readily obtainable inspections.

Table IV. Comparison of Estimated and Determined Heats of Combustion of Gasolines

Gasoline Sample	Weight % of Hydrogen	Heat of Combustion Determined	Heat of Combustion Estimated	Divergence
1	15.3	20,430	20,350	− 80
2	15.2	20,460	20,340	−120
3	15.1	20,400	20,300	−100
4	14.9	20,304	20,240	− 64
5	14.3	19,988	20,020	+ 32
6	14.1	20,003	19,980	− 23
7	14.0	19,885	19,950	+ 65
8	14.0	19,866	19,950	+ 84
9	13.9	19,930	19,900	− 30
10	13.8	19,867	19,880	+ 13
11	13.6	19,840	19,830	− 10
12	13.4	19,814	19,750	− 64
13	13.3	19,655	19,720	+ 65
14	13.2	19,640	19,690	+ 50
15	12.9	19,581	19,600	+ 19
16	12.5	19,499	19,450	− 49
17	12.5	19,420	19,450	+ 30
18	12.0	19,355	19,310	− 45
19	10.5	18,732	18,810	+ 78
			Av.	− 8

Table III. Heats of Combustion of Pure Hydrocarbons Boiling in the Gasoline Range

(Selected values from literature)

Hydrocarbon	Molecular Weight	Boiling Point	Hydrogen	Heat of Combustion[a] Gross		Net	Reference
		° C.	Weight %	° K., cal./mole	B. t. u./lb.	B. t. u./lb.	
Paraffins							
n-Pentane	72.15	36.0	16.77	845.27	21,100	19,515	*24*
Isopentane	72.15	28.0	16.77	843.36	21,051	19,466	*15*
Neopentane	72.15	9.5	16.77	840.61	20,983	19,398	*15*
n-Hexane	86.18	68.8	16.37	1002.4	20,948	19,400	*24*
2-Methylpentane	86.18	60.2	16.37	998.54	20,867	19,319	*16*
2,3-Dimethylbutane	86.18	58.1	16.37	993.9	20,770	19,222	*14*
n-Heptane	100.20	98.4	16.10	1149.7	20,664	19,142	This paper
2-Methylhexane	100.20	90.0	16.10	1148.9	20,650	19,128	*14*
2,3-Dimethylpentane	100.20	89.7	16.10	1148.0	20,633	19,111	5
2,2,3-Trimethylbutane	100.20	80.9	16.10	1147.9	20,631	19,109	*14*
n-Octane	114.22	125.6	15.88	1316.4	20,754	19,252	*24*
2-Methylheptane	114.22	117.2	15.88	1306.1	20,591	19,089	*14*
2,4-Dimethylhexane	114.22	109.9	15.88	1298.4	20,470	18,968	*16*
2,2,4-Trimethylpentane	114.22	99.2	15.88	1303.40	20,549	19,047	*17*
n-Nonane	128.25	150.7	15.72	1473.4	20,688	19,201	*24*
2-Methyloctane	128.25	142.8	15.72	1454.1	20,417	18,930	*16*
2,3-Dimethylheptane	128 25	140.6	15.72	1458.8	20,483[b]	18,996	*14*
2,2,5-Trimethylhexane	128.25	124.1	15.72	1458.8	20,483[b]	18,996	*14*
2,2,4,4 - Tetramethylpentane	128.25	122.3	15.72	1458.8	20,483[b]	18,996	*14*
Naphthenes							
Cyclopentane	70.13	49.5	14.38	783.6	20,121	18,761	*14*
Methylcyclopentane	84.16	71.8	14.38	937.5	20,062	18,702	7
1,2 - Dimethylcyclopentane	98.18	91.9	14.38	1096.0	20,102	18,742	3
1 - Methyl - 2 - ethylcyclopentane	112.21	121.0	14.38	1250.4	20,067[b]	19,707	*14*
Propylcyclopentane	112.21	131.4	14.38	1250.4	20,067[b]	19,707	*14*
Cyclohexane	84.16	80.8	14.38	939.0	20,095	18,735	*25*
Methylcyclohexane	98.18	100.3	14.38	1091.4	20,018	18,658	*18*
1,1 - Dimethylcyclohexane	112.21	119.9	14.38	1242.5	19,939	18,579	*18*
1,3 - Dimethylcyclohexane	112.21	122.7	14.38	1238.0	19,867	18,507	*18*
Ethylcyclohexane	112.21	131.6	14.38	1250.4	20,067[b]	18,707	*14*
1,1,3 - Trimethylcyclohexane	126.23	138.7	14.38	1394.7	19,896	18,536	*14*
Aromatics							
Benzene	78.11	80.1	7.75	782.0	18,030	17,298	*6*
Toluene	92.13	110.8	8.76	934.2	18,261	17,433	*14*
o-Xylene	106.16	144.0	9.50	1090.9	18,506	17,608	*28*
m-Xylene	106.16	139.3	9.50	1090.9	18,506	17,608	*28*
p-Xylene	106.16	138.4	9.50	1087.1	18,441	17,543	*28*
Ethylbenzene	106.16	136.2	9.50	1089.0	18,473	17,575	*18*
1,3,5-Trimethylbenzene	120.19	164.6	10.06	1242.8	18,622	17,670	*28*
1,2,4-Trimethylbenzene	120.19	169.2	10.06	1239.8	18,577	17,625	7
Propylbenzene	120.19	159.5	10.06	1245.7	18,666	17,714	*28*
Olefins							
Pentene-1	70.13	30.2	14.38	806.78[g]	20,581[c]	19,221	*26*
2-Methylbutene-2	70.13	38.4	14.38	795.7	20,431	19,071	*18*
Hexene-1	84.16	63.6	14.38	963.9[g]	20,477[c]	19,117	*26*
2-Methylpentene-2	84.16	67.3	14.38	950.8	20,346[b]	18,986	*14*
Heptene-1	98.18	94.9	14.38	1120.9[g]	20,410[c]	19,050	*26*
5-Methylhexene-1	98.18	84.7	14.38	1107.1	20,305[b]	18,945	*14*
Octene-1	112.21	122.5	14.38	1277.9[g]	20,378[c]	19,018	*26*
2,4,4 - Trimethylpentene-1	112.21	101.2	14.38	1263.4	20,275[b]	18,915	*14*
Nonene-1	126.23	145.3	14.38	1434.9[g]	20,345[c]	18,985	*26*
3-Methyloctene-1	126.23	136.3	14.38	1419.7	20,252[b]	18,892	*14*

[a] Heating values of liquid hydrocarbons unless designated by *g*.
[b] Calculated values; considered to be accurate within 1%.
[c] Values shown are calculated for liquid hydrocarbons.

Acknowledgments

The authors desire to express their sincere thanks to A. Voorhies, Jr., and J. A. Hinckley for their helpful suggestions.

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Temperature Coefficient of Density and Refractive Index for Hydrocarbons in the Liquid State

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Using the equation

$$d_t/4 = d20/4 + \alpha(t - 20) + \beta(t - 20)^2$$

a single curve of α *vs.* molecular weight and a single curve of β *vs.* molecular weight have been found applicable to all types of hydrocarbons with very few exceptions. From these curves the temperature coefficient of density of a liquid hydrocarbon at atmospheric pressure can be predicted from the molecular weight within ±0.00002 gram per cc. per ° C. The only definite exceptions to this relation are benzene, pentaethylbenzene, and hexapropylbenzene which show coefficients 0.00010 gram per cc. per ° C. higher than the predicted values. The curves are also applicable to hydrocarbon mixtures.

This relation is more general than any previously published for predicting the temperature coefficient of density. The corresponding relationship between compressibility and molecular weight is being investigated.

Since Ward and Kurtz have shown that for hydrocarbons

$$\Delta n = 0.60\ \Delta d$$

an accurate method of calculating density change also provides a satisfactory method for calculating change in refractive index.

THE change of volume and density of hydrocarbons with change of temperature has been the subject of a number of careful investigations. The commonly accepted equation for change of density with temperature is

$$d_t/4 = d20/4 + \alpha(t - 20) + \beta(t - 20)^2 \qquad (1)$$

where α is the temperature coefficient, $\Delta d/\Delta t$, of the liquid density, d, at 20° C., and one atmosphere pressure, and β covers the variation of $\Delta d/\Delta t$ with temperature t. The problem has been to correlate α and β with other properties, so that change of density with temperature can be predicted.

Early work in the field by Bearce and Peffer (*2*) was based on a plot of α and β against density. Recently Beale (*1*) proposed a new attack by plotting the cubical coefficient of expansion against boiling point. Paraffins, naphthenes, and aromatics form lines of similar curvature based on the data used by Beale. This type of plot is convenient, since cubical coefficient at 20° C. × density at 20° C. equals $\Delta d/\Delta t$.

Ward and Kurtz (*22*) derived a plot of density coefficient *vs.* density at 20° C. using a plot of cubical coefficient of expansion *vs.* boiling point, and a boiling point–density plot. The plot of density *vs.* density coefficient (Figure 11, *22*) reveals separate and very different lines for different hydrocarbon types. It is known that the naphthene lines on this graph will require some revision, but that the paraffin and aromatic lines are essentially correct.

The National Standard Petroleum Oil Tables used in the petroleum industry for correcting the specific gravity of oils for temperature change are based on a single curve of α *vs.* density, and a single curve of β *vs.* density. Since each hydrocarbon type has a curve of its own, the accuracy of a single curve for hydrocarbon mixtures is seriously dependent on composition. Jessup (*10*) confirmed this when he found that two oils of the same specific gravity but from different sources differed more than 30 per cent in their thermal expansion. Figure 11 of Ward and Kurtz (*22*) shows the aromatics, especially those of low molecular weight, to be widely divergent from the curve corresponding to the National Standard Petroleum Oil Tables.

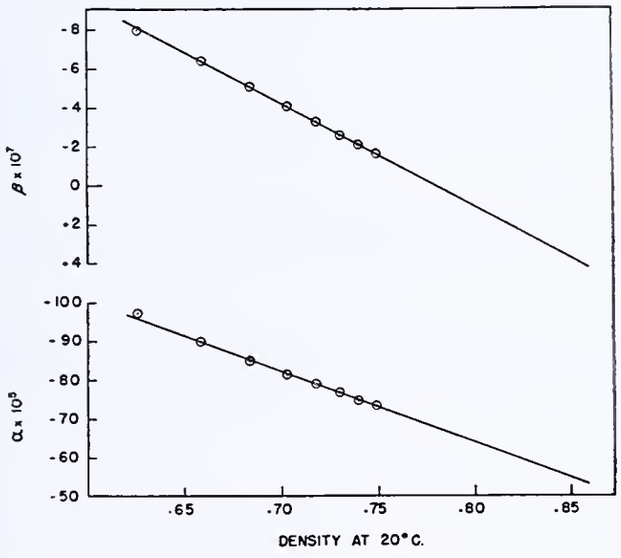


FIGURE 1. TEMPERATURE-DENSITY COEFFICIENTS *vs.* DENSITY FOR *n*-PARAFFINS

Calingaert, Beatty, Kuder, and Thomson (*3*) developed a correlation between α and β and number of carbon atoms for the normal and branched paraffins up to 20 carbon atoms. The present work is an amplification of their work and provides a single correlation between α and β and molecular weight for hydrocarbons of any structure, and applies to all data now available, including compounds of high molecular weight. Using this correlation the change of density with temperature can usually be predicted within ±0.00002 gram per cc. per ° C.

The derivation of the general relation between molecular weight and the α and β coefficients is simple. The smoothed values of α and β for the normal paraffins of 5 to 12 carbon atoms, which have been calculated by Calingaert and coworkers (*3*) from the data of Dornte and Smyth (*4*), were plotted against density, d20/4. As shown in Figure 1 very good straight lines were obtained. Consideration of physical property relations made it seem probable that a plot of α and β against molecular weight might give a single relation for both paraffins and naphthenes, and perhaps for other classes of hydrocarbons. The equation of Calingaert and coworkers (*3*) relating molecular weight to molecular volume and density was, therefore, used to convert the density co-

ordinate in Figure 1 to molecular weight. In order to correct the resulting curve so that it would be representative of average paraffins rather than just normal paraffins, the data of Edgar and Calingaert (*5*) were used. These data indicate that the average $\Delta d/\Delta t$ for all nine isomeric heptanes is 1.5×10^{-5} unit higher than the value for the corresponding normal compound. The α coordinate of the graph of molecular weight *vs.* α was, therefore, shifted 1.5×10^{-5} unit. The graph thus obtained is shown as Figure 2 and numerical values for constructing such a graph are given in Table I.

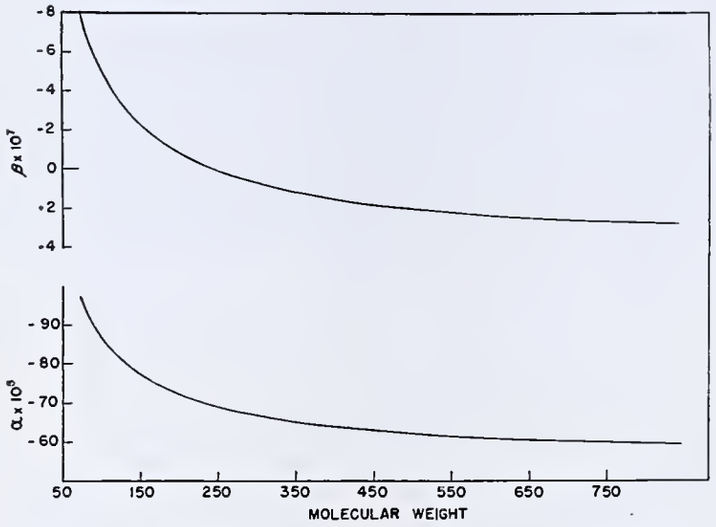


Figure 2. Temperature-Density Coefficients *vs.* Molecular Weight for Hydrocarbons

In the derivation of Figure 2 the equation of Huggins (*9*), relating molecular weight to molecular volume for the normal paraffins, can be used in place of the Calingaert equation. If this is done the curves obtained are identical with those shown in Figure 2 up to 450 molecular weight, but there is a small increasing deviation above this point, which at 700 molecular weight amounts to 1×10^{-5} in α and 2×10^{-8} in β. Since neither Calingaert nor Huggins claims accuracy for his density–molecular weight relations above 282 molecular weight (20 carbon atoms), and since the data for the higher molecular weight pure compounds are not sufficient to provide completely definite empirical evidence, we cannot yet be sure which relation is best. The authors have arbitrarily used the Calingaert relation in this work.

Having obtained a relation between the α and β coefficients and molecular weight for paraffins as shown in Figure 2 and Table I, it was necessary to determine the accuracy of this generalization for paraffins, and whether or not it could be used for other classes of hydrocarbons.

Examination of density data (*6*, *7*, *11*) at the two temperature extremes for all types of hydrocarbons on which densities are reported at two temperatures, at least 20° C. apart, provided the data which are presented in Table II. Inspection of Table II makes it clear that by using Equation 1 and Figure 2 the change of density with temperature can be calculated accurately for paraffins, naphthenes, aromatics, and a variety of unsaturated compounds. [Francis (*8A*) gives an equation for the density coefficient of paraffins which is essentially in agreement with this paper.] The average calculated density change for 98 compounds for an average temperature change of 74° C. is correct within ±0.0012 gram per cc., and the average calculated density coefficient agrees with the experimentally determined density coefficient within ±0.00002 gram per cc. per ° C. This is about ±3 per cent uncertainty on either the density change or the temperature coefficient. Furthermore, there is little systematic error since the deviation of the average (taking sign into consideration) is zero.

Table I. Data Necessary for Construction of Curves of α and β Coefficients Plotted against Molecular Weight

Molecular Weight	$\alpha \times 10^5$	$\beta \times 10^7$
72[a]	−97.4	−8.1
85	−91.5	−6.4
100	−86.7	−5.0
120	−82.0	−3.7
140	−78.5	−2.6
160	−75.8	−1.8
180	−73.7	−1.2
200	−71.9	−0.7
225	−70.3	−0.2
250	−69.0	+0.2
275	−67.9	+0.5
300	−66.8	+0.8
350	−65.2	+1.3
400	−63.8	+1.6
450	−62.8	+1.9
500	−62.0	+2.1
600	−60.8	+2.5
700	−60.1	+2.7
800	−59.5	+2.8

[a] Curve not recommended for less than 72 molecular weight.

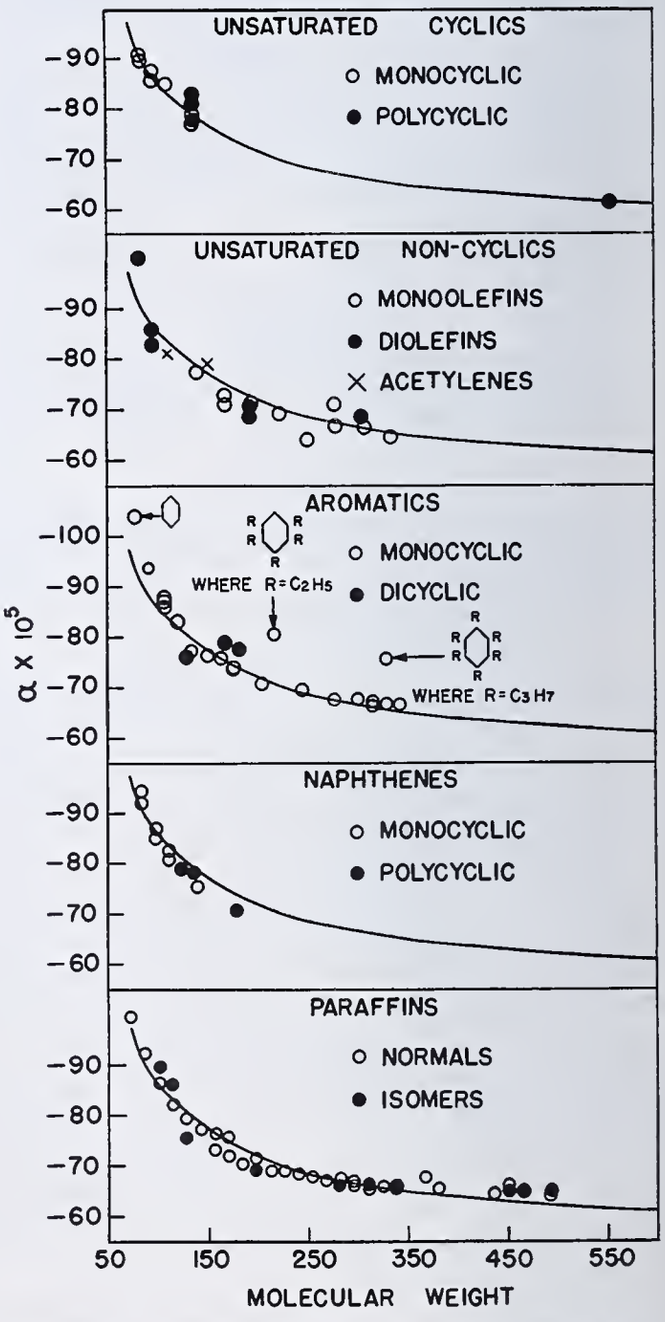


Figure 3. Agreement of Derived and Observed α for Hydrocarbons

Table II. Comparison of Calculated and Experimental Change of Density

Hydrocarbon Type	No. of Compounds	Average Temperature Range, ° C.	Average Deviation Regardless of Sign		Deviation of Average Taking Sign into Account		Maximum Deviation of Any Single Compound	
			Δd^a	$\frac{\Delta d}{\Delta t} \times 10^5$	Δd^a	$\frac{\Delta d}{\Delta t} \times 10^5$	Δd^a	Corresponding Deviation of $\frac{\Delta d}{\Delta t} \times 10^5$
Paraffins	39	94	0.0015	2	+0.0004	0	+0.0065	+3
Naphthenes								
Mono	6	55	0.0010	2	−0.0008	−1	−0.0019	−3
Poly	3	59	0.0012	2	−0.0012	−2	−0.0021	−3
Aromatics								
Mono	18[b]	62	0.0005	1	+0.0002	0	+0.0011	+3
Di	3	67	0.0023	4	+0.0001	+1	−0.0033	−4
Olefins								
Mono	9	69	0.0012	2	−0.0008	−1	−0.0047	−5
Di	6	48	0.0017	4	−0.0001	−1	+0.0032	+7
Acetylenes	2	55	0.0013	3	−0.0001	0	−0.0014	−3
Unsaturated cyclics	12	62	0.0007	1	−0.0001	0	+0.0030	4
All hydrocarbons	98	74	0.0012	2	0	0	+0.0065	+3

[a] $\Delta d = (d_{t_1} - d_{t_2}$ observed) − $(d_{t_1} - d_{t_2}$ calculated).
[b] Benzene, toluene, pentaethylbenzene, and hexapropylbenzene omitted from average.

Only a few compounds show poor agreement with the average data. Benzene, pentaethylbenzene, and hexapropylbenzene are badly out of line ($+10 \times 10^{-5}$ gram per cc. per ° C.). Toluene is slightly out of line ($+ 4 \times 10^{-5}$ gram per cc. per ° C.). Some of the large rings synthesized by Ruzicka are also out of line, while others check very well. Additional precise data on such compounds are needed. All the other data are in good agreement. The above-mentioned compounds were omitted from the average in Table II. All other reliable data, where the data cover an appreciable temperature range, have been included, and the worst deviations in each group have been indicated in the last two columns of Table II. The worst deviations in density change in the paraffin and naphthene groups correspond to ±0.00003 gram per cc. per ° C., or about ±5 per cent on the coefficient. The worst deviation in any group is about 10 per cent on the coefficient and probably represents poor data.

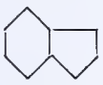
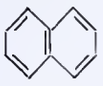
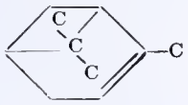
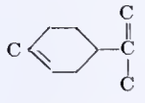
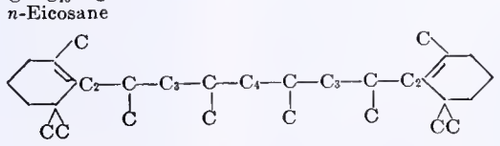
A further cross check on the accuracy of Equation 1 and Figure 2 was obtained by assuming that the β curve was correct, and taking all the difference between observed and calculated density change as error in the α coefficient. These observed α values (assuming β is correct) are plotted in Figure 3 for the same data as shown in Table II. Figure 3 makes it very clear that with the exception of the four aromatic compounds already mentioned, the agreement obtained is excellent and there are no systematic deviations.

One rather surprising thing about the data for paraffins which is shown by Figure 3 is that the normal paraffin data agree very exactly with the curve, although in its derivation a distinction between normal and average paraffins was made, and the curve was adjusted to agree with what the authors believe to be the best data for average paraffins. At any rate the final curve obtained (Figure 2) has excellent empirical justification, and is recommended for normal paraffins as well as for all other hydrocarbons.

The question may be raised, that compounds of unusual structure may not agree with this general system of correcting density. Table III was, therefore, prepared to show that for a specific group of varied compounds the agreement is good—that is, approximately ±0.001 gram per cc. or less for 50° C. change in temperature.

Also, using the limiting density of Kurtz and Lipkin (*15*) for the paraffin homologous series, and Figure 1 corrected for the isomer effect, we obtain a value of $\Delta d/\Delta t$ at 20° C. = 5.5×10^{-5} gram per cc. per ° C. The best estimate of data for hydrogenated rubber indicates $\Delta d/\Delta t$ to be 5.7×10^{-5} gram per cc. per ° C. which checks the limiting value satisfactorily.

Table III. Density Change with Temperature for Some Interesting Compounds

	Molecular Weight	Temperature Range, ° C.	Deviation Δd^a	Deviation $\frac{\Delta d}{\Delta t}$
C—C_3—C *n*-Pentane	72	120	+0.0028	+0.00001
 Cyclooctane	112	58	−0.0005	−0.00001
 Hydrindane	124	58	−0.0009	−0.00002
 Naphthalene	128	97	−0.0033	−0.00003
 *dl*-α-Pinene	136	66	−0.0005	−0.00001
 *d*-Limonene	138	167	+0.0006	+0.00001
C=C—C_{13}—C Hexadecene-1	224	65	−0.0003	−0.00001
C—C_{18}—C *n*-Eicosane	281	98	0.0000	0.00000
 Octadecahydrocarotene	555	51	0.0001	0.00000

[a] $\Delta d = (d_{t_1} - d_{t_2}$ observed) − $(d_{t_1} - d_{t_2}$ calculated).

Table IV. Validity of Equation 1 for Calculating Densities at Temperatures Ranging from Melting to Boiling Point

Temperature	*n*-Heptane Deviation		2,2-Dimethylpentane Deviation	
	Δd^a	$\frac{\Delta d}{\Delta t} \times 10^5$	Δd^a	$\frac{\Delta d}{\Delta t} \times 10^5$
° C.				
−120			+0.0072	+5
−110			+0.0060	+5
−90	+0.0004	0	+0.0039	+4
−70	−0.0001	0	+0.0024	+3
−50	−0.0003	0	+0.0013	+2
−30	−0.0006	−1	+0.0003	+1
−10	−0.0005	−2	−0.0001	0
+10	0	0	−0.0001	−1
+20	...	...	...	...
+30	−0.0001	−1	−0.0001	−1
+50	−0.0001	0	−0.0004	−1
+70	0	0	−0.0009	−2
+80			−0.0015	−2
+90	0	0		

[a] Δd = (d20/4 − d_4^t observed) − (d20/4 − d_4^t calculated) when t is 20° C. or higher.

Δd = (d_4^t observed − d20/4) − (d_4^t calculated − d20/4) when t is less than 20° C.

One last point which deserves consideration is the ability of Equation 1 to fit the intermediate points in a density-temperature curve as well as the extreme points. In Table IV data are given for *n*-heptane and 2,2-dimethylpentane at temperatures ranging between the melting and boiling points, using the data for Dornte and Smyth (*4*) and Smyth and Stoops (*21*), respectively. The data for *n*-heptane between −90° and +90° C. agree within ±0.0005 gram per cc., but the observed deviations for 2,2-dimethylpentane are considerably larger than for *n*-heptane. Smyth and coworkers published data for eleven compounds between the melting point and boiling point, 2,2-dimethylpentane being the worst. The average deviation for all points of all 11 compounds is ±0.0009 gram per cc. These data and the data of Table II indicate that one should expect errors on the average of ±0.001 in density when correcting over a temperature range of 50° to 75° C.

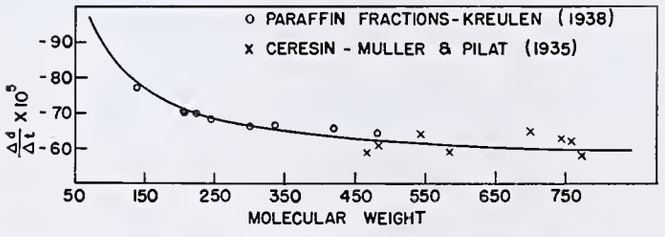


Figure 4. Agreement of Derived and Observed $\Delta d/\Delta t$ for Paraffin and Ceresin Fractions

The authors feel that the proposed method of correcting densities will be sufficiently accurate for practically all density corrections for pure compounds except in the case of the few aromatic compounds specifically mentioned, which are all highly symmetrical and mononuclear.

The question of hydrocarbon mixtures is under investigation. For the present it is recommended that Equation 1 and Figure 2 be used in the same manner as for pure hydrocarbons. However, the average molecular weight should be calculated on a mole per cent basis from the molecular weight and percentage of each constituent, or should be determined experimentally. It is recognized that molecular weight is not an easily determined property, but it may be estimated from correlations of molecular weight with other more easily determined physical properties such as density and viscosity (*19*). It is probable that the data of Jessup (*10*), which correlate thermal expansion factors with viscosity and specific gravity, are basically a correlation of coefficient of expansion with molecular weight.

Figures 4 and 5 show the agreement of thermal expansion data for hydrocarbon mixtures—(i. e., petroleum cuts). In Figure 4 are data on the paraffin fractions of Kreulen (*13*) and ceresin fractions of Muller and Pilat (*20*). The two investigators reported $\Delta d/\Delta t$ without giving any temperature range. Since $\Delta d/\Delta t$ can vary only a few per cent from α, depending on the temperature range and the sign and magnitude of β, the authors feel justified in plotting these $\Delta d/\Delta t$ values on the α *vs.* molecular weight curve. These data agree very well.

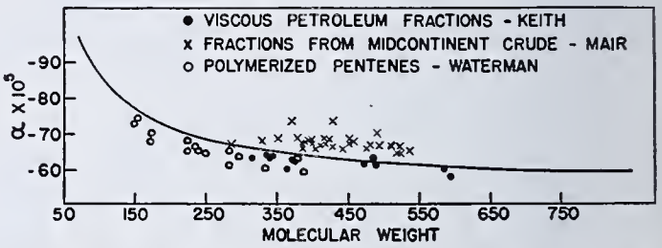


Figure 5. Agreement of Derived and Observed α for Hydrocarbon Fractions

Figure 5 presents the data of three different laboratories on hydrocarbon fractions on which molecular weight and densities over a range of 40° C. or more are reported. Mair and Schicktanz (*17*) and Mair and Willingham (*18*) report data on mid-continent crude which has been separated into fractions in connection with their composition studies. Keith and Roess (*12*) report on viscous petroleum cuts which have also been separated according to boiling point and composition. Waterman, Leendertse, and Makkink (*23*) report on polymerized pentene fractions before and after hydrogenation. The data fall on both sides of the derived curve for α, assuming β is correct, and any one laboratory falls systematically on one side of the line or the other. Since the temperature coefficient of density is sensitive to small errors in either density or temperature, the deviations are probably caused by systematic experimental errors.

Additional precise data for temperature coefficients are needed. For the present, the authors feel that the curve of α *vs.* molecular weight is the best that can be drawn in view of the discrepancies between the data of various investigators. It is probable that it will be necessary to revise this curve slightly above 400 molecular weight when more precise data have become available.

Since it was shown by Ward and Kurtz (*22*) that a very simple relation

$$\Delta n = 0.60\ \Delta d \qquad (2)$$

exists between the increment of refractive index and the increment of density, it is possible by using Figure 2 and Equations 1 and 2 to calculate the change in refractive index corresponding to a given change in density with sufficient accuracy for most purposes. More complete equations, such as the Sellmeier-Drude equation with the ϕ coefficient (*22*, Equation 4) or the Eykman equation (*8*, *14*, *16*), are recommended for more precise correlation of density change with refractive index change.

The relation between the α and β coefficients and molecular weight developed in this paper suggests that there should be a similar relation involving compressibility coefficients. Jessup (*10*) supports this with the evidence that oils of the same specific gravity and viscosity (which also means the same molecular weight) have the same compressibility. This will be investigated.

Acknowledgment

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Consistency Test for Lubricating Greases

Counterbalance Modification for Soft or Liquid Greases

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THE American Society for Testing Materials provides a method for determining the consistency of lubricating greases (*1*), by which consistency is expressed as a number indicative of the depth (mm./10) to which the standard double-pitched grease cone penetrates the sample in a free-falling period of 5 seconds, the total weight of the grease cone and the other moving parts attached to it being 150 grams. Numerous objections have been advanced to this method of test. Yet despite the fact that it is empirical and its results do not, and perhaps cannot, express fundamental properties of the grease in absolute units, the test has, nevertheless, been very useful as a manufacturing control and as a guide in purchase specifications.

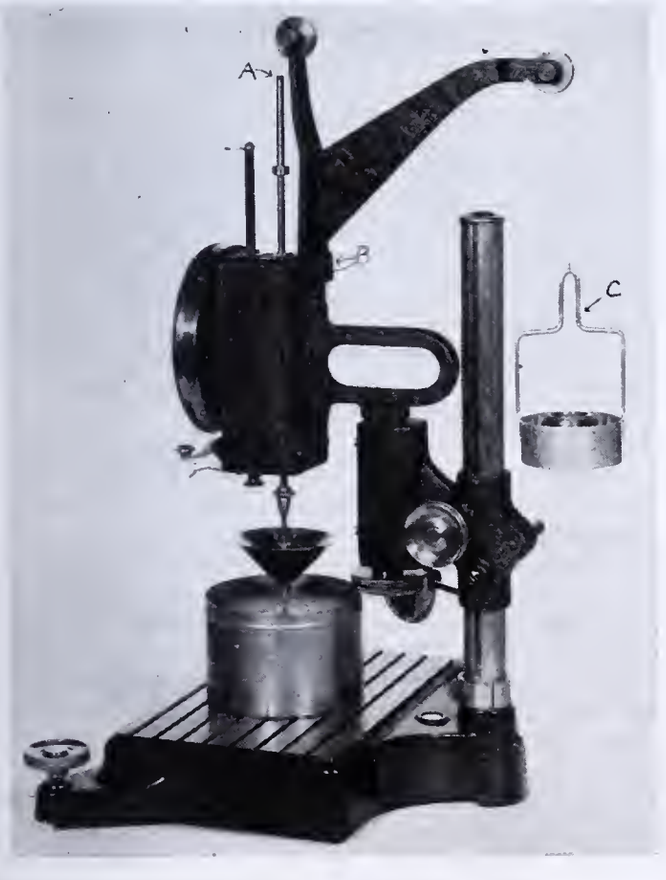

Figure 1. Penetrometer Assembled and Equipped with Counterbalancing Device

Greases generally are plastic substances; hence, their resistance to flow is of a twofold nature and, unlike viscosity, cannot be expressed by a single coefficient. When a stress, no matter how small, is applied to a viscous liquid, a permanent deformation results; with a plastic substance the stress must exceed a certain minimum. Other methods of test for more fundamentally expressing the plastic character of greases and similar substances are available. Among these should be mentioned the plastometer based on the work of Bingham and Green (*3*, *8*), the constant-sheer viscometer of Arveson (*2*), and more recently the consistometer of Blott and Samuel (*4*). The Stormer and MacMichael torsion type viscometers have also been proposed for this purpose.

The method of test which is the subject of this paper has been developed and used as an extension of the A. S. T. M. method to greases too soft for the standard A. S. T. M. procedure. The stated scope of the A. S. T. M. method—measuring the unworked or the worked consistency of lubricating greases which have a worked consistency less than 400—generally limits its use to greases no softer than No. 0 cup grease.

Greases much softer than this are regularly manufactured for lubricating washing machines, mine car journal bearings, rail curves, etc., and the consistencies or fluidities of such products have been customarily estimated in a number of ways, varying from appearance and feel through measurements by Saybolt, Stormer, and MacMichael viscometers, and by the use of special instruments such as the buret con-

sistometer (*9*), the mobilometer (*6*, *7*), and falling sphere-type instruments (*5*, *10*).

It was considered desirable to work with the standard A. S. T. M. apparatus and method as far as possible. The proposed method of test involves the standard A. S. T. M. apparatus and procedure, with the addition of an easily removable attachment by means of which the force applied to the grease can be reduced to any desired quantity.

Figure 1 shows a penetrometer assembled and equipped with the counterbalancing device, which is fixed to the back of the penetrometer by means of two screws that enter the holes provided for securing the back plate of the original penetrometer. The modification is encompassed by *A*, a punctured screw to secure the thread; *B*, screws to secure the counterbalancing arm; and *C*, an aluminum cup to hold the counterbalancing weights. The two pulleys over which the thread passes are held between jeweled bearings. It can be installed or removed in a few minutes, although it does not interfere with regular A. S. T. M. penetrations when the thread is disconnected from the top of the shaft of the penetrometer.

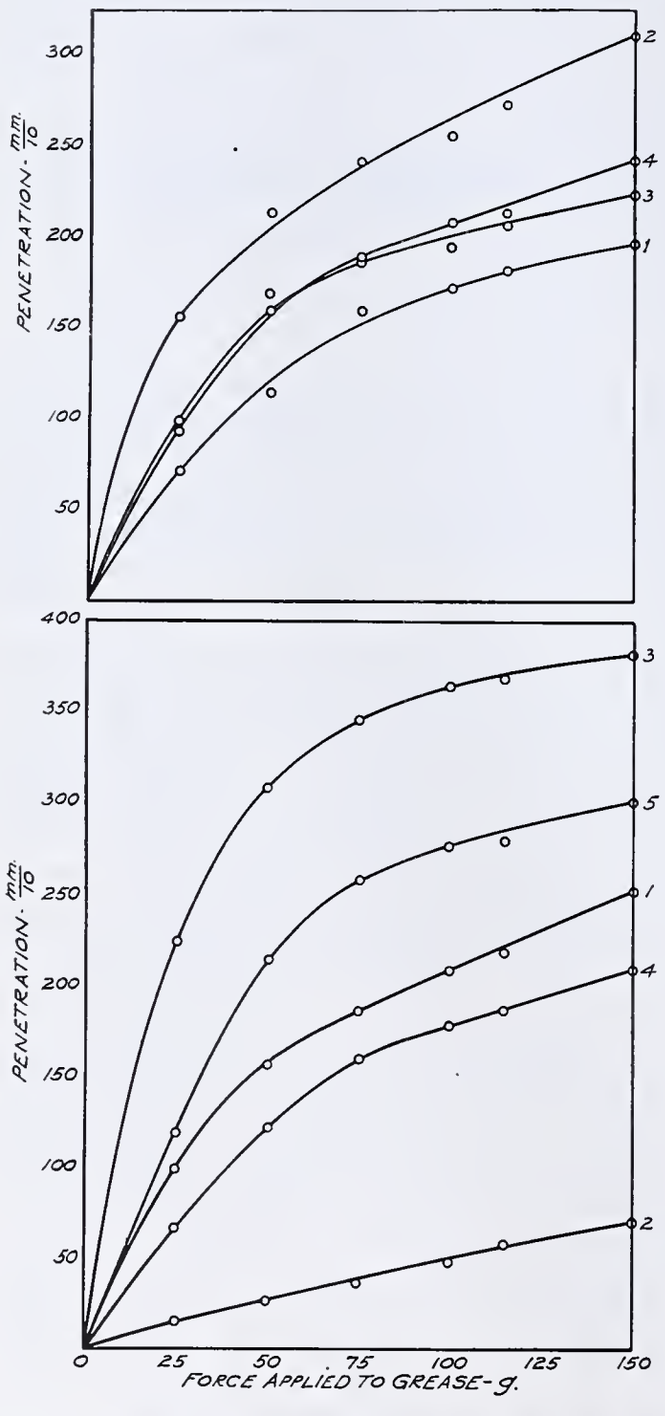

FIGURE 2. EFFECT OF WEIGHT ON EXTENT OF PENETRATION
Above. Lime-soap greases
Below. Soda-soap sponge or fiber greases

TABLE I. RESULTS BY A. S. T. M. AND PROPOSED METHODS COMPARED

	Consistency at 77° F.	
	A. S. T. M.	Proposed method
Force applied to sample, grams	150	25
Lime-soap cup grease		
Sample A	312	156
Sample B	235	95
Sample C	243	93
Sample D	196	72
Soda-soap sponge or fiber grease		
Sample A	383	225
Sample B	300	119
Sample C	208	67
Sample D	27	15
Fluid lime-soap grease	Too soft	366
Fluid soda-soap grease	Too soft	350
Cylinder stock	Too soft (viscosity Saybolt Universal at 210° F. 265 sec.)	355
Blown rapeseed oil	Too soft (viscosity Saybolt Universal at 210° F. 1638 sec.)	324
Crude residuum	Too soft (viscosity Saybolt Furol at 210° F. 100 sec.)	308

In this manner the standard A. S. T. M. cone and attached moving parts are counterbalanced by means of a thread-arm-pulley-cup arrangement to reduce the force applied to the grease from the standard 150 grams to any desired lesser weight. It was found that 25 grams is a satisfactory minimum, less tending to give erratic results apparently due to unavoidable friction. Various types of fiber, horsehair, and catgut were tested in an effort to find a practical one with which friction will be low, and silk thread (Belding Heminway, pure silk A) was found most suitable.

To conduct a test, weights are put into the aluminum cup to counterbalance the usual moving part to any desired extent. From this point the test is conducted exactly in the manner of the A. S. T. M. standard procedure.

In Figure 2 are shown typical curves for penetration *vs.* force acting on lime-soap greases and soda-soap greases. These indicate that the results by the proposed method are not related in a simple manner to those obtained by the A. S. T. M. standard method (150 grams), precluding the possibility of expressing the former in terms of the latter. For this reason it is recommended that results of the proposed test be expressed in the customary manner except that the number be followed with (counterbalance, 25), indicating that the counterbalance procedure was employed with a net force of 25 grams.

Though it would be possible from several tests on a soft grease, employing different weights, to calculate the penetration in terms of the standard A. S. T. M. method, it is not believed necessary for the present purposes.

On several occasions it was found that the friction of the moving parts in the proposed apparatus increased, owing to small misalignment of the pulleys or excessive tightening of the bearings caused by jarring of the apparatus, and was reflected in lower penetrations. For this reason it is necessary to have a criterion by which the apparatus may be periodically checked and adjusted to give the highest penetration, which means minimum friction under the particular test condition. Having attained that adjustment, the apparatus is considered to be in condition to test a batch of samples. A crude petroleum residuum of 100 seconds Saybolt Furol viscosity at 210° F. (98.9° C.) has been found to be a particularly suitable reference material.

The precision of the proposed test meets the requirements of the standard A. S. T. M. method.

In Table I are given, for comparison, typical results obtained by the standard A. S. T. M. procedure and this proposed modification, both at the standard temperature of 77° F. (25° C.).

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Analytical Procedure for Mixtures of Organic Sulfur Compounds

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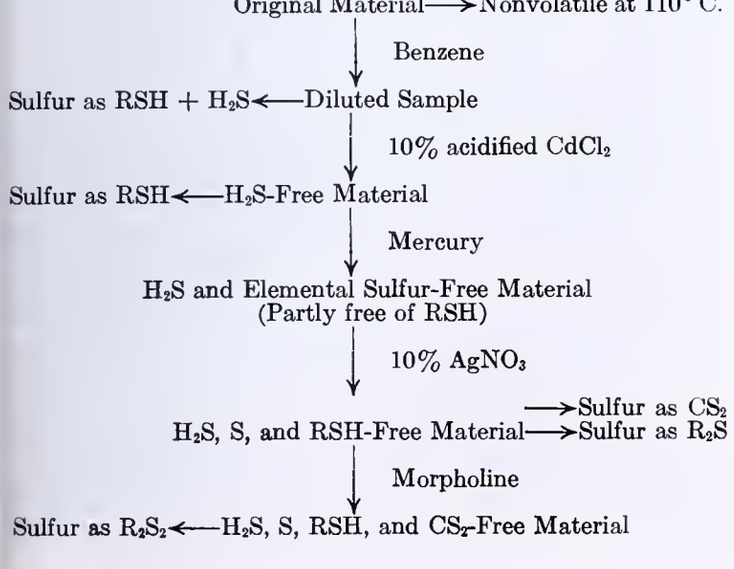
ANALYSES of mixtures of organic sulfur compounds were encountered requiring the development of reliable analytical methods which could be carried out in logical sequence. Preliminary qualitative analyses of these mixtures indicated hydrogen sulfide, mercaptans, free sulfur, carbon disulfide, and organic sulfides as the chief components. Tests on many mixtures showed no hydrocarbons present, and either no disulfides or only very small amounts.

While complete credit is not claimed for all the individual methods used, the procedure as a whole, including the use of benzene as the diluent, and the modifications of procedure and technique in the various methods employed are worthy of special note because of their value in making a rapid and accurate analysis of a mixture containing the above compounds.

Procedure

The undiluted liquid sample was tested qualitatively for the presence of hydrogen sulfide by shaking a small amount in a test tube with an excess of acidified cadmium chloride solution (*2*), a yellow precipitate indicating the presence of hydrogen sulfide. If a precipitate formed the material was filtered and the filtrate was shaken with a globule of clean mercury (*3*). Any darkening of the mercury indicated the presence of free sulfur.

Schematic Diagram of Procedure. Horizontal arrows indicate a determination. Vertical arrows indicate a separation or dilution.



Six to eight grams of the original liquid were accurately weighed from a ground-glass joint dropping bottle into a 500-cc. volumetric flask containing at least 300 cc. of c. p. benzene. The volumetric flask was stoppered immediately and tipped several times to ensure rapid solution. The dropping bottle was weighed again as rapidly as possible. Benzene was added to the flask up to the 500-cc. mark and the solution was thoroughly mixed.

If hydrogen sulfide was present, the diluted solution was transferred from the volumetric flask to a 1-liter separatory funnel and shaken for at least 5 minutes with 50 cc. of 10 per cent acidified cadmium chloride solution. The lower layer of cadmium chloride solution was discarded and the benzene solution was washed three successive times with 50 cc. of distilled water. The benzene solution was then filtered through a No. 42 Whatman filter paper into another separatory funnel, and 25-cc. portions of this hydrogen sulfide–free material were withdrawn by a pipet for the determination of sulfur as mercaptan according to Method I (*2*).

If no hydrogen sulfide was present the mercaptan sulfur was determined directly on the diluted sample. When hydrogen sulfide was desired quantitatively, the sum of sulfur as hydrogen sulfide and mercaptan was determined directly on the diluted sample using Method I; hydrogen sulfide was then removed with cadmium chloride solution as above and subsequently the sulfur as mercaptan was determined on the hydrogen sulfide–free material. The difference between these two determinations represented the per cent of sulfur as hydrogen sulfide.

If free sulfur was present, the remainder of the hydrogen sulfide–free material was shaken for at least 10 minutes with 50 cc. of clean mercury to precipitate free sulfur as mercuric sulfide. The mercury was drawn off with as much mercuric sulfide as possible.

If the mercaptan sulfur was over 1 per cent on the basis of the original material, the mercaptans were removed by shaking the unfiltered material in the separatory funnel with 25 cc. of 10 per cent silver nitrate solution. After washing with three successive 50-cc. portions of water, the material was filtered through a No. 42 Whatman filter paper into a glass-stoppered bottle. To a 150-cc. portion of this filtrate in a separatory funnel were added 50 cc. of a 1 to 1 solution of morpholine and absolute ethyl alcohol and the mixture was intimately mixed.

After centrifuging for 15 minutes, the supernatant liquid was decanted through a No. 41 Whatman filter paper into a 500-cc. separatory funnel where it was washed three successive times with 50-cc. portions of distilled water, or until the washings were no longer alkaline to litmus. The morpholine-alcohol-free solution was used for the determination of sulfur as disulfides by Method II (*3*) after its mercaptan sulfur content was determined by Method I.

Separate portions of the hydrogen sulfide–free and elementary sulfur–free material in the glass-stoppered bottle were taken for the determination of sulfur as carbon disulfide by Method III (*4*) and as sulfide by Method IV (*1*, *5*).

Method I

Determination of Per Cent of Sulfur as Mercaptans (also suitable for per cent of sulfur as hydrogen sulfide or combined per cent of sulfur as mercaptans and as hydrogen sulfide). Twenty-five cubic centimeters of sample were measured by a pipet into a 250-cc. glass-stoppered iodine flask containing 15 cc. of ferric alum indicator solution (6 grams of ferric ammonium sulfate dissolved in 100 cc. of a 1 to 1 nitric acid solution and diluted to 2 liters with alcohol) and an accurately measured excess quantity of 0.05 *N* silver nitrate solution. The mixture was shaken vigorously for at least 1 minute and the excess silver nitrate was titrated with 0.05 *N* ammonium thiocyanate to a salmon-pink end point. The per cent of sulfur as mercaptans was calculated on the basis of the original sample.

Method II

Determination of Per Cent Sulfur as Disulfides. Exactly 100 cc. of sample were mixed with 50 grams of glacial acetic acid and 25 grams of powdered zinc in a 250-cc. Erlenmeyer flask, which was immediately attached to a reflux condenser equipped at the top with a trap containing an accurately measured amount of 0.05 *N* silver nitrate solution, and diluted with a sufficient amount of distilled water (about 25 cc.) so that the tip of the outlet tube from the top of the condenser was just below the surface of the solution. The zinc–acetic acid mixture was refluxed slowly for 3 hours, after which it was cooled to room temperature before detaching the flask from the condenser. The mixture was decanted from the caked zinc into a 500-cc. separatory funnel and the flask and caked zinc were washed several times with distilled water, the washings (total of about 75 cc.) being added to the separatory funnel. The acetic acid–water solution was drawn off from the benzene solution and discarded.

The benzene was washed with successive portions of distilled water until the washings were no longer acid to litmus, after which it was filtered through No. 42 Whatman filter paper into a glass-stoppered bottle. Twenty-five cubic centimeters of the reduced and filtered benzene solution were taken for a mercaptan sulfur determination, following the procedure in Method I. The excess silver nitrate in the trap was titrated with 0.05 *N* ammonium thiocyanate solution as in Method I and the amount of silver nitrate which reacted with low-boiling mercaptans that escaped condensation was determined. The cubic centimeters of silver nitrate used by the trap were added to the cubic centimeters of silver nitrate used by the reduced benzene solution before calculating the amount of sulfur as disulfides in the original sample.

Method III

Determination of Per Cent Sulfur as Carbon Disulfide. Ten cubic centimeters of diluted sample were measured by pipet into a 250-cc. iodine flask containing 10 cc. of a fresh solution of potassium hydroxide in either absolute ethanol or methanol. The solution was gently swirled for at least 2 minutes and then neutralized with glacial acetic acid to the phenolphthalein end point. One cubic centimeter of fresh starch solution was added and the xanthic acid was titrated immediately with 0.1 *N* iodine solution. It was found that the xanthic acid solution could not be allowed to stand before titration with the iodine solution. During the titration with iodine the solution became milky white and the end point was observed when 1 drop of iodine solution turned the solution tan. As a further check on the end point, 100 cc. of water were added to the tan solution and the color was observed. Usually the purple color of starch in the presence of iodine became evident at once. If not, one more drop of iodine solution was always sufficient for a deep blue color. Duplicate titrations checked well within 0.05 cc. and were usually within 0.02 cc. or identical. The per cent of sulfur as carbon disulfide was calculated on the basis of the original sample.

Method IV

Determination of Sulfur as Sulfides. Fifty cubic centimeters of diluted sample were measured by a pipet into a 500-cc. separatory funnel containing 50 cc. of distilled water and an excess of bromine water; usually 10 cc. sufficed. The mixture was shaken violently for at least 10 minutes, after which 10 cc. of a 15 per cent potassium iodide solution were added and the mixture was shaken for 10 to 15 seconds; the liberated iodine was destroyed by adding a sufficient quantity of 0.1 *N* solution of sodium thiosulfate and again shaken for 10 to 15 seconds. The mixture was allowed to stand for 10 minutes to allow the two layers to separate and the lower water layer was drawn off into a 500-cc. Erlenmeyer flask. The tip of the separatory funnel was washed out carefully with water. The benzene solution was washed three successive times with 50-cc. portions of distilled water, the tip of the separatory funnel being washed out each time, and all washings were added to the solution in the Erlenmeyer flask. The water solution in the Erlenmeyer flask was titrated with 0.1 *N* potassium hydroxide solution, using methyl red indicator. The sulfur as sulfides was calculated to the basis of the original sample.

Method V

Per Cent of Nonvolatile Material at 110° C. Six to eight grams of the original liquid material were weighed from a dropping bottle into a porcelain crucible that had previously been dried at 110° C. and weighed. Evaporation was carried out slowly on top of a steam bath and when complete the crucible was placed in an oven at 110° C. for 30 minutes. The crucible was allowed to cool in a desiccator and then weighed. The difference in weight represented the nonvolatile material at 110° C and consisted almost entirely of elementary sulfur.

Discussion

The results of many analyses made by the above procedure and methods show that approximately 5 per cent of material is lost or otherwise unaccounted for at room temperature. Calculating the mercaptan and sulfide sulfur to the methyl derivatives and the sulfur as carbon disulfide to carbon disulfide, the total per cent varied from 95 to 100 per cent.

During the early part of this investigation it was found that secondary low-octane reference fuel was unsuitable for dilution purposes because the presence of a small amount of unsaturates appreciably interfered with the determination of sulfur as sulfides and as carbon disulfide.

When determining sulfur as carbon disulfide (Table I) it was found that the procedure of refluxing the alcoholic potassium hydroxide with the sample to form the xanthate was not only unnecessary but undesirable because it gave erroneous results, and that if free sulfur was not removed before forming the xanthate the results were far too high. In addition, if mercaptans are present in an amount more than 1 per cent of mercaptan sulfur, the xanthate procedure gives erroneous results.

Identical results were obtained with potassium hydroxide in either absolute ethanol or methanol, when freshly prepared. Samples of c. p. carbon disulfide weighed out, diluted, and determined according to the above procedure gave results of 84.21 per cent sulfur as carbon disulfide, the exact theoretical value for pure carbon disulfide. In this case, however, there were no filtrations necessary after removal of other constituents and hence no loss.

Table I. Effect of Time of Xanthate Formation on Carbon Disulfide Determination

Time of Contact *Seconds*	Sulfur as CS_2 %
30	78.07
60	81.13
120	81.64
240	81.64

If the free sulfur was removed with metallic mercury prior to the determination of the mercaptan sulfur, the mercaptan sulfur was always low. This is attributed to some compound formation or adsorption of the mercaptan with the mercuric sulfide. This phenomenon is the subject of another paper from this laboratory which will be published shortly. For example, in one sample, before treating the diluted material with mercury to remove free sulfur, the mercaptan sulfur was 0.077 per cent, and after removal of the free sulfur the mercaptan sulfur was 0.036. For this reason the authors' procedure is to determine the mercaptan sulfur prior to the removal of free sulfur; they have found that free sulfur does not interfere with the mercaptan sulfur determination.

That the determination of sulfur as sulfide was perfectly satisfactory was made evident by the analysis of a synthetic

sample (Table II), the composition of which was unknown to the operator. It was found essential to shake the material with bromine water at least 10 minutes; otherwise the per cent sulfide sulfur would be low.

It has been found that if the disulfide sulfur of the original material is over 1 per cent, it interferes appreciably with the determination of the sulfide. In such cases, the H_2S—, S—, RSH—, and CS_2—free material is reduced with zinc and acetic acid as described above. The mercaptan formed is removed with silver nitrate before determining the sulfides on the residue.

Table II. Analysis of Unknown Synthetic Sample

Sulfur as:	S Found %	S Present %	Compound	Found %	Present %
RSH	0.00	0.00	CH_3SH	0.00	0.00
CS_2	70.18	72.42	CS_2	83.34	86.00
R_2S	6.70	6.71	$(CH_3)_2S$	12.98	13.00
Elementary	0.98	0.99	S	0.98	0.99
			Total	97.30	99.99

Since the quantity of hydrogen sulfide present is dependent on the efficiency of stabilization and is not of primary importance, it was not determined quantitatively in the majority of cases. The percentages of sulfur as carbon disulfide, sulfide, nonvolatile material at 110° C. (nearly all elementary sulfur), and mercaptan included 98 to 100 per cent of the total material present and were of primary importance. No difficulty was experienced with black silver sulfide in observing the end point of Method I for determining hydrogen sulfide. All that was necessary was to allow the benzene and aqueous layers to separate well before observing the color in the aqueous layer.

Table III. Results Obtained on Various Samples

Sulfur as H_2S %	Sulfur as RSH %	Sulfur as CS_2 %	Sulfur as R_2S %	Sulfur as R_2S_2 %	Nonvolatile at 110° C. %	Total (R = CH_3) %
..	0.16	79.40	1.52	..	..	97.47
..	0.02	79.33	0.06	..	..	94.33
..	0.00	80.54	0.12	..	..	95.76
..	0.08	66.23	10.10	0.09	..	98.46
..	..	80.24	...	..	..	95.29
..	..	80.59	...	..	..	95.70
..	0.15	79.88	0.20	..	..	95.48
..	0.05	80.30	0.14	..	..	95.73
..	0.00	72.42	6.70	..	..	96.32
..	0.18	79.02	0.70	..	..	95.47
..	0.26	79.13	0.72	..	..	95.76
..	0.41	76.98	1.03	..	0.33	94.36
..	0.35	77.78	1.80	..	0.30	96.68
..	0.04	74.12	0.42	..	5.27	94.16
..	0.23	79.42	0.82	..	0.89	97.14
0.04	0.02	78.51	1.02	..	0.04	95.37

If the mercaptan sulfur was over 1 per cent on the basis of the original material, the mercaptans were removed by shaking the diluted sample with 25 cc. of 10 per cent silver nitrate solution followed by washing with three successive 50-cc. portions of water. Over 1 per cent of mercaptan sulfur interfered with the carbon disulfide determination. The removal of mercaptans was accomplished before filtering the mercury sulfide from the removal of elementary sulfur in order to avoid another filtration, and thus a loss by evaporation.

In all the titration methods used there was the advantage of back-titration if the end point were overrun. The whole procedure has been found rapid, accurate, and practical, and has been in use for over a year. Table III gives typical results by this procedure on a number of routine samples.

The 5 per cent discrepancy in the total analysis is believed to be due to loss by evaporation through handling and filtration. This was shown by checking the composition of the liquid samples by specific gravities and fractional distillations as well as chemical tests. If as much as 5 per cent of other possible constituents had been present it would have been evident through these tests. Furthermore, when using synthetic samples of pure carbon disulfide and following this procedure, but without the filtrations and shaking required when other constituents are present, the values obtained were within 0.1 per cent of the theoretical for sulfur as carbon disulfide.

The method described is not limited to the low-boiling materials for which it was developed. When handling higher boiling materials no evaporation losses should be experienced during the several filtrations. Furthermore, if there is dilution with paraffinic material this method is still applicable. The two limitations involved are the complete absence of unsaturates, and dilution with a suitable solvent to a nonviscous state.

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Viscosity Measurement

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IN A PREVIOUS paper (*3*) simple and accurate viscometers were described for the measurement of the viscosities of liquids ranging from one third to more than one thousand times the viscosity of water. These instruments are now in extensive use throughout the world in the petroleum and chemical industries. It is the purpose of this paper to describe a further modification of the Ostwald viscometer which is particularly suited for measuring the viscosities of opaque liquids. It is also valuable in obtaining rate of shear–shearing stress data that are useful in studies relating to asphalts, wax crystallization, and oil-flow properties at low temperatures. In addition, the transition region between viscous flow and plastic flow may be investigated with the viscometer described here. Hence it should find application in studying plastic liquids in general.

A sketch of the viscometer, very similar to that previously described (*3*), is shown as Figure 1. Since it is designed for opaque liquids the efflux bulbs are on the bottom. When testing, the liquid flows from reservoir *A* into the pear-shaped 3-cc. efflux bulbs on the bottom; hence the meniscus is always in contact with clean unwetted glass and can be seen.

Operation of Viscometer

The viscometer is loaded by holding it in an inverted vertical position with the capillary side submerged in the test liquid. Suction is then applied to the other arm of the viscometer by a piece of rubber tubing to the mouth of the operator or to a water aspirator. The main reservoir, *A*, is filled and the liquid is brought into the capillary to the etched line just below *A*. The excess liquid is wiped off the end of the capillary arm and the instrument is inclined slightly to cause the oil to flow by gravity from the upper capillary into *A;* this small amount of liquid will be discharged into the main capillary leading to bulb *B*. A short piece of rubber tubing with a pinch clamp closing one end is then slipped over the open end of the upper capillary, which can then be turned to a vertical position and placed in the con-

stant temperature bath. When the test temperature is reached the pinch clamp is removed and the liquid will flow by gravity into the three bulbs at the lower end of the instrument.

The first bulb which the liquid enters at the base of the capillary is for the purpose of allowing some liquid to discharge from the upper reservoir, *A*, and thus the diameter of the meniscus in this bulb is large throughout the test. This reduces both the effect of loading errors and surface tension corrections. As the liquid passes the etched line between bulbs *B* and *C* a stop watch is started and the time for the meniscus to reach the etched line between bulbs *C* and *D* is noted. At this point a second stop watch is started and the time for the meniscus to reach the etched line above bulb *D* is recorded. From each efflux time the viscosity is obtained by multiplying the respective viscometer constants by the respective efflux time. The viscosities so computed should agree. The constants for *C* and *D* are accurately established by calibration with liquids whose viscosities are accurately known. Equipment and procedure for calibration are explained in detail elsewhere (*1*, *4*, *5*).

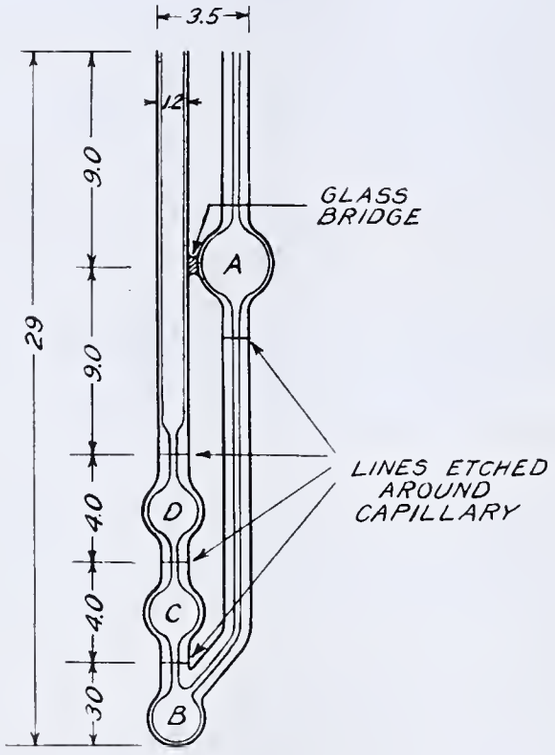

FIGURE 1. VISCOMETER FOR OPAQUE LIQUIDS AND SHEARING RATE STUDIES

Dimensions in cm.

Shearing Rate Studies

As is well known, the viscosity of a true liquid or Newtonian fluid is independent of shearing rate. The presence or absence of plastic or semiplastic flow is conveniently obtained from shearing rate studies in which rate of shear in reciprocal seconds is plotted against shearing stress as dynes per square centimeter. In a Newtonian liquid such data yield a straight line passing through the origin using ordinary coordinates. The slope of such a line is inversely proportional to the viscosity of the fluid. In semiplastic flow a correlation of rate of shear–shearing stress data usually does not give a straight line. Such a line or curve may or may not go through the origin.

The definition of such shearing rate–shearing stress curves is conveniently obtained with this viscometer. Thus the character of the fluid under investigation is capable of better definition. Various externally applied pressures are easily applied, so that the fluid flows through the capillaries at different rates of shear. Depending upon the fluid and the viscometer dimensions, any suitable driving head may be applied to the viscometer arm by inert gas pressure, which is readily measurable on a manometer.

Such shearing rate studies are important in defining the flow characteristics of lubricating oils in the vicinity of their cloud and pour points. The A. S. T M. cloud and pour points of an oil afford only limited knowledge of the actual flow behavior of an oil at low temperatures. The use of pour-point depressors or wax-crystallization inhibitors has further complicated the significance and reliability of the routine pour-point tests of oils. A reasonable amount of work carried out at this college on the flow behavior of oils at low temperatures shows clearly the utility of a viscometer of this sort in studies relating to dewaxing, wax crystallization, pour-point depressors, low-temperature flow characteristics, and low-temperature storage of oil. Viscometers of the type described here have proved very useful in such investigations. They naturally yield much more significant and useful conclusions than are obtained simply from the cloud and pour-point data alone.

Magnitude and Source of Errors

This instrument is very similar to those previously described (*3*) and is superior to them only for measurements of the viscosity of opaque liquids and for measuring the effect of shearing stress on viscosity. The method of calculating the various corrections is identical to the methods given in the previous publication and need not be repeated here. The summation of the various errors is below 0.3 per cent.

The precision of measurements with this instrument when measuring viscosities of opaque liquids is ±0.2 per cent. The accuracy depends upon the accuracy to which the absolute viscosity of water is known at 20° C., since water at this temperature is used as the ultimate reference basis for calibration work. This error in the viscosity of water at 20° C. is probably in the neighborhood of 0.5 per cent. However, relative viscosities are very accurate and through the efforts of the American Society of Testing Materials the petroleum industry uses 1.007 centistokes as the kinematic viscosity of water at 20° C. This means that all relative viscosity measurements have the same reference basis.

The instruments described here can be constructed to cover any desired viscosity range by calculating the diameter of the capillary from Poiseuille's law. A charge of approximately 13 cc. is required. If desired, the dimensions of the instrument can be changed to require a charge of 5 cc., by reducing the size of the various bulbs and the capillary diameter. It is also possible to include a small bulb between the various measuring bulbs on the bottom, so that the operator does not have to stop one clock and start another at the same time.

Comparison with Other Viscometers

Anderson, Wright, and Griffin (*2*) have described an instrument designed for viscosity measurements on opaque liquids. Their instrument appears to be well designed and will undoubtedly yield accurate results. The instrument presented as Figure 1, however, is considerably simpler in design and therefore less expensive.

The Saybolt viscometer is used to determine viscosities of opaque liquids. However, the results are empirical and a large quantity of test liquid (90 cc.) is necessary. It does not lend itself to the application of external pressures for shearing rate studies without introducing serious errors.

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Quality Tests for Petroleum Solvent Naphthas

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PETROLEUM solvent naphthas were first supplied, as such, to replace turpentine in varnishes and paints. Called "mineral spirits", then as now, they were cut to conform with the evaporation rate and flash point of turpentine. As their use increased, they were followed by a faster evaporating fraction, called V. M. & P. naphtha, which provided a new tool for paint formulation. Later, shortly after the turn of the century, other light fractions were found useful in dry cleaning, rubber cements, and vegetable oil extraction, so that prior to the World War a half dozen more or less standardized naphthas were regularly available.

Not until comparatively recently, however, have petroleum solvent naphthas achieved a general acceptance by the protective coatings industry. This hesitance was due largely to a notorious lack of uniformity among naphthas, both in shipments of a single product and between items offered as strictly competitive. Efforts to correct this situation came from purchaser and supplier alike, and have resulted in a number of adequate quality tests.

"Quality", in connection with petroleum products, usually connotes the degree of useful composition, or the ability to pass tests which guarantee performance—e. g., octane rating. Tests for purity, such as noncorrosiveness, are assumed as being met by such highly refined fractions as modern solvent naphthas, and thus the quality of a solvent naphtha is normally determined from inspections of its gravity, volatility, and solvency properties.

Use of the term "solvency" has recently been questioned (*23*). It has been pointed out that the behavior of otherwise similar naphthas may vary somewhat between different types of solutes, and that therefore such a term as "resin solvency" is more rigorously correct. Similarly, a naphtha's aromatic content, long accepted as a good measure of solvency, has now been interpreted in terms of resin solution viscosity, and a formidable equation derived for "aromatic coefficient" (*10*). Solvency, however, is commonly used to rate one quality of a naphtha, and is employed in this paper.

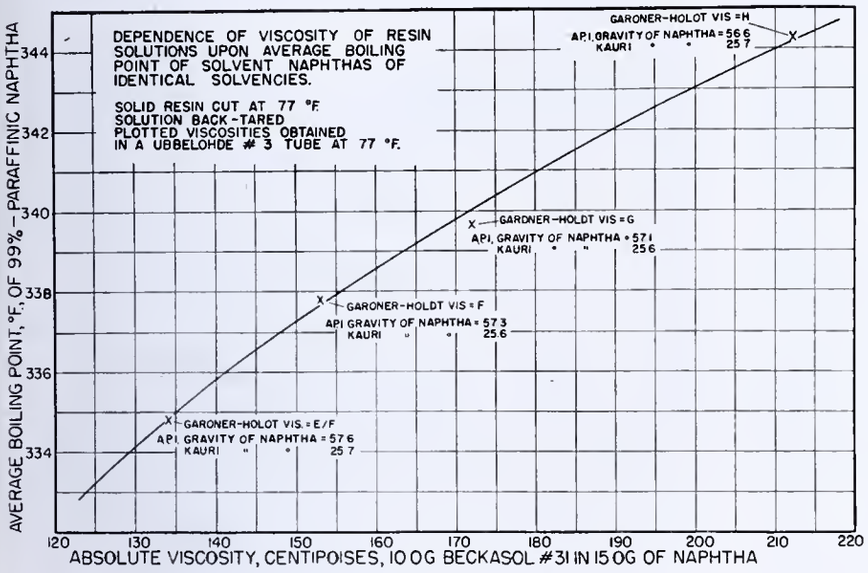

FIGURE 1

Volatility

The rate at which a naphtha leaves a film of a protective coating, or the pores of a solid, depends upon the evaporation rate of the naphtha and upon its rate of diffusion through the film or solid. "Solvent retention" varies widely, particularly among the several types of protective coating vehicles, and is best determined by drying-time measurements (*5*, *13*, *15*). Evaporation rate of the naphtha alone may be relatively determined by a number of existing methods (*4*, *6*, *12*, *13*, *14*, *16*, *17*, *24*, *26*, *29*, *35*, *36*, *38*, *40*, *43*, *44*), most of which are still confined to their parent laboratories. Some simple, inexpensive, precise, and accurate method for evaporation rate has long been sought by both manufacturers and consumers of naphthas, and would doubtless be adopted by a majority of their laboratories immediately after its final development. Meanwhile, volatility is controlled by the A. S. T. M. distillation, which not only involves temperatures at variance with those encountered in the actual use of solvent naphthas, but also fails to recognize the divergence in latent heats of vaporization between naphthas of similar boiling ranges and intermediate fill—e. g., between paraffinic and aromatic thinners.

Development of Solvency Tests

The first measure of solvency to be adopted by the petroleum industry was aniline

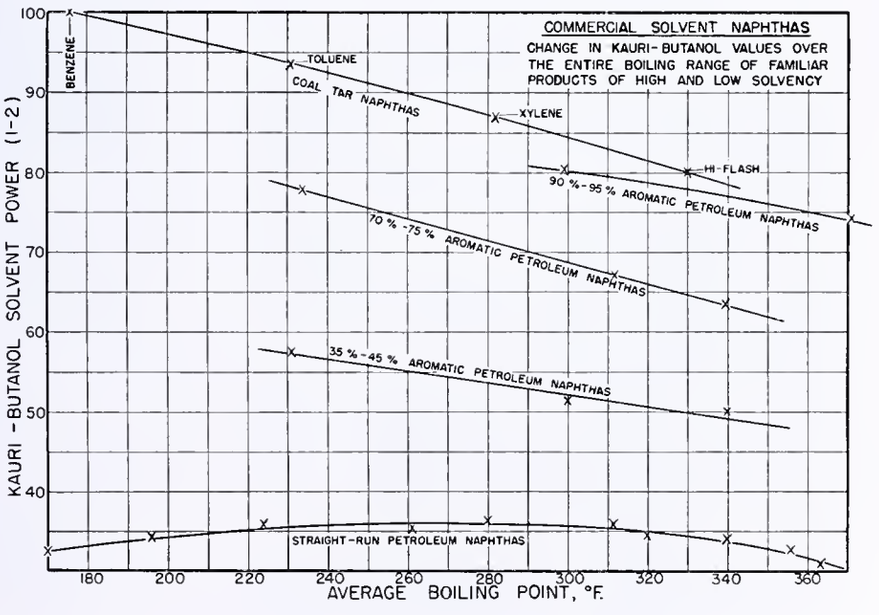

FIGURE 2

point (*41*). It remains as the only method which does not require any temperature control. Originally inapplicable to highly aromatic naphthas, because of the comparatively high freezing point of aniline, it has fortunately been supplemented by "mixed aniline point", wherein the aromatic naphtha is diluted with an equal volume of a paraffinic naphtha whose aniline point is 60° C. (*9*, *25*).

Earliest solvency test to be widely accepted by the paint and varnish industry was kauri-butanol value (*22*). It has proved so valuable that a number of refinements have been contributed by both the supplier (*1*, *2*, *3*) and consumer (*31*, *32*, *34*) industries, and standardized kauri solutions may be purchased ready for use. As might be expected, it has inevitably run afoul of an occasional supplier who felt it rated his product comparatively lower in solvency than did his favorite method, so that for a time—particularly among those who failed to appreciate its breadth of coverage or understand its correct interpretation—the fashion was to preface a discussion of solvency with a round condemnation of kauri-butanol value. The test survives, however, in both its original and modified forms, as by far the most popular quick appraisal of solvency.

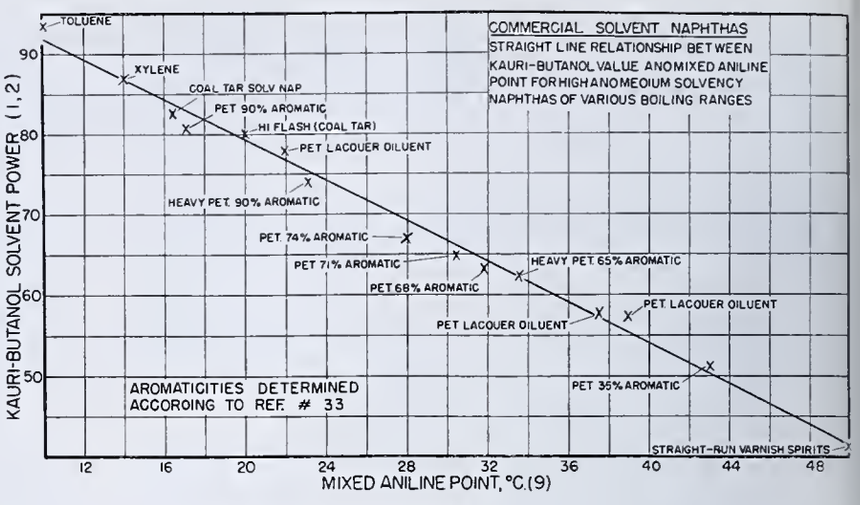
COMMERCIAL SOLVENT NAPHTHAS
STRAIGHT LINE RELATIONSHIP BETWEEN KAURI-BUTANOL VALUE AND MIXED ANILINE POINT FOR HIGH AROMEOIUM SOLVENCY NAPHTHAS OF VARIOUS BOILING RANGES
TOLUENE
XYLENE
COAL TAR SOLV NAP
PET 90% AROMATIC
HI FLASH (COAL TAR)
PET LACQUER DILUENT
HEAVY PET 90% AROMATIC
PET 74% AROMATIC
PET 71% AROMATIC
PET 68% AROMATIC
HEAVY PET 65% AROMATIC
PET LACQUER DILUENT
PET LACQUER DILUENT
PET 35% AROMATIC
STRAIGHT-RUN VARNISH SPIRITS
AROMATICITIES DETERMINED ACCOROING TO REF. # 33
KAURI-BUTANOL SOLVENT POWER (1,2)
MIXED ANILINE POINT, °C.(9)

FIGURE 3

Second in rank among the so-called "dilution ratio" tests is nitrocellulose dilution ratio, or "diluting power". Efforts have been made toward standardization (*7*, *11*, *18*), but the test is commonly restricted to the evaluation of lacquer diluents.

Other dilution ratio tests employ synthetic resins. Either the resin is dissolved in an oxygen-containing solvent and titrated to a cloud point with the hydrocarbon thinner, or a solution of the resin in a high-solvency naphtha is titrated to cloud with a reference naphtha of high aniline point—e. g., the 60° C. aniline point naphtha mentioned above. Such tests may be uniquely appropriate to the formulation of a given resin or naphtha, but the host of synthetics available, together with the constant improvements and changes within each type, render difficult any attempt to select one synthetic and hope for the adoption of a test built around it. General acceptance of any particular viscosity-solvency test is similarly hindered by the same circumstance.

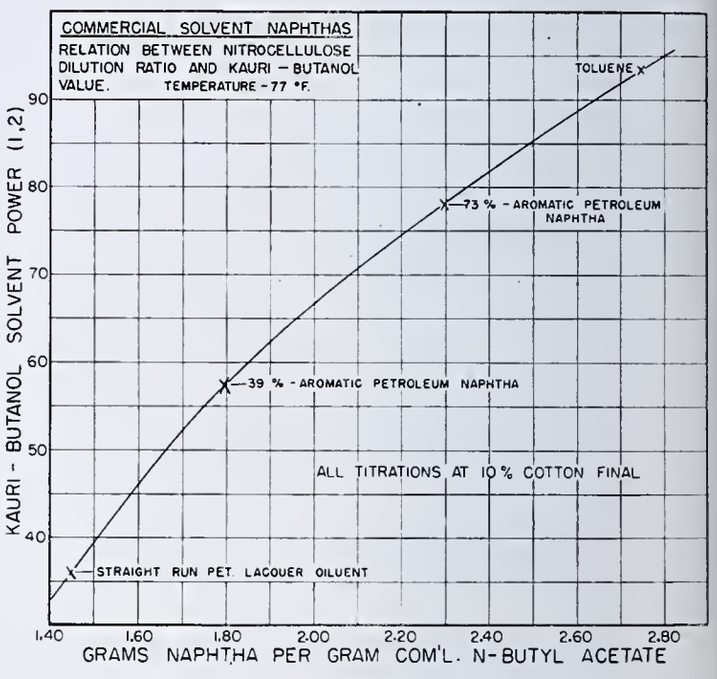
COMMERCIAL SOLVENT NAPHTHAS
RELATION BETWEEN NITROCELLULOSE DILUTION RATIO AND KAURI - BUTANOL VALUE. TEMPERATURE - 77 °F.
TOLUENE
73 % - AROMATIC PETROLEUM NAPHTHA
39 % - AROMATIC PETROLEUM NAPHTHA
ALL TITRATIONS AT 10 % COTTON FINAL
STRAIGHT RUN PET. LACQUER DILUENT
KAURI - BUTANOL SOLVENT POWER (1,2)
GRAMS NAPHTHA PER GRAM COM'L. N-BUTYL ACETATE

FIGURE 4

Physical Property Tests

Physical properties which rate solvency, and which are deemed important to petroleum and paint products, include gravity, viscosity, refractive index, and surface tension. Gravity provides a rough measure of uniformity, as long as the product is derived from the same source stock. Viscosity (Figure 9) is seldom run on a solvent naphtha itself, since costly equipment and skilled manipulation are required to obtain significant results (*8*, *42*) and the relation between the viscosity of a naphtha and the viscosity of its solution of, say, a synthetic resin, is a complex one (*45*). Refractive index and surface tension, however, are measured in more familiar apparatus, and are occasionally designated as specification tests. The former is associated with specific dispersion, a recommended measure of aromaticity (*20*, *21*, *28*). Upon refractive index is also based the most accurate and precise method of proximate analysis yet developed (*27*, *30*, *33*).

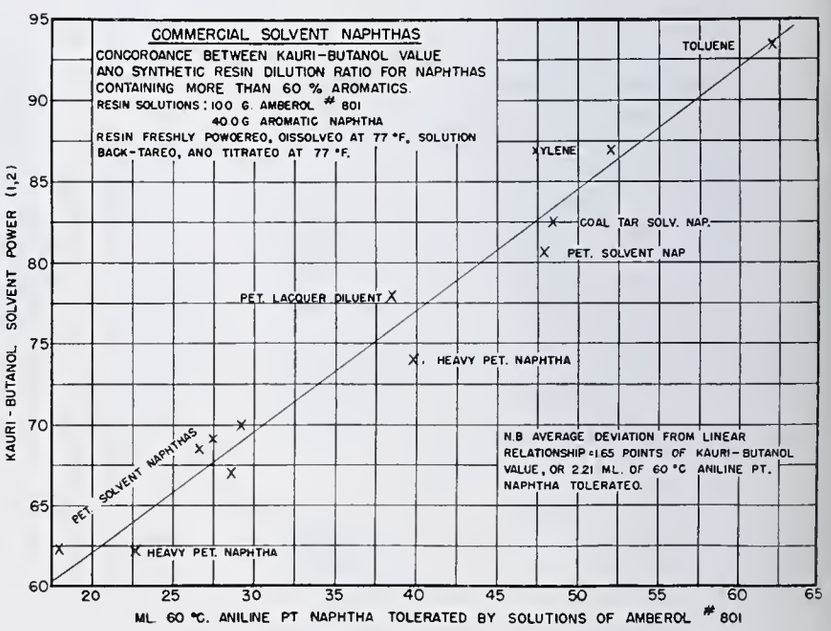
COMMERCIAL SOLVENT NAPHTHAS
CONCORDANCE BETWEEN KAURI-BUTANOL VALUE AND SYNTHETIC RESIN DILUTION RATIO FOR NAPHTHAS CONTAINING MORE THAN 60 % AROMATICS.
RESIN SOLUTIONS: 100 G. AMBEROL # 801
400 G AROMATIC NAPHTHA
RESIN FRESHLY POWDERED, DISSOLVED AT 77 °F. SOLUTION BACK-TARED, AND TITRATED AT 77 °F.
TOLUENE
XYLENE
COAL TAR SOLV. NAP.
PET. SOLVENT NAP
PET. LACQUER DILUENT
HEAVY PET. NAPHTHA
PET. SOLVENT NAPHTHAS
HEAVY PET. NAPHTHA
N.B AVERAGE DEVIATION FROM LINEAR RELATIONSHIP = 1.65 POINTS OF KAURI-BUTANOL VALUE, OR 2.21 ML. OF 60 °C ANILINE PT. NAPHTHA TOLERATED.
KAURI-BUTANOL SOLVENT POWER (1,2)
ML. 60 °C. ANILINE PT NAPHTHA TOLERATED BY SOLUTIONS OF AMBEROL # 801

FIGURE 5

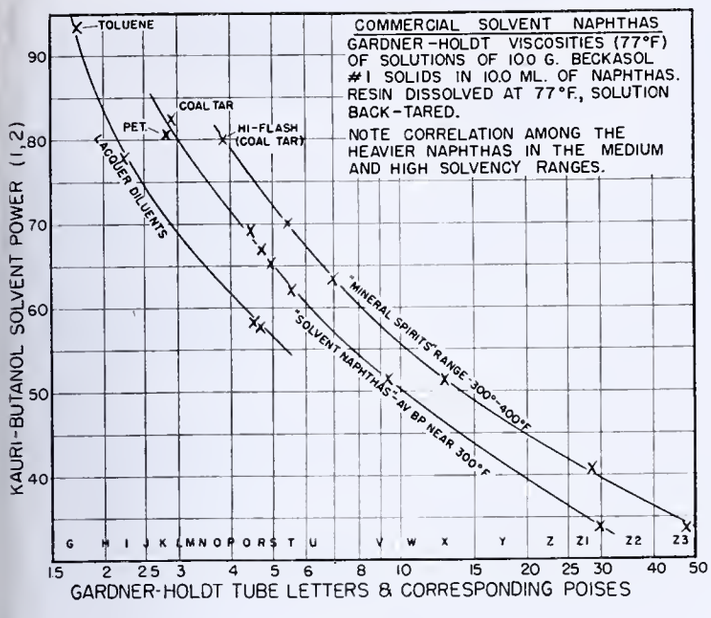
COMMERCIAL SOLVENT NAPHTHAS
GARDNER-HOLDT VISCOSITIES (77°F) OF SOLUTIONS OF 100 G. BECKASOL #1 SOLIDS IN 100 ML. OF NAPHTHAS. RESIN DISSOLVED AT 77°F., SOLUTION BACK-TARED.
NOTE CORRELATION AMONG THE HEAVIER NAPHTHAS IN THE MEDIUM AND HIGH SOLVENCY RANGES.
TOLUENE
COAL TAR
PET.
HI-FLASH (COAL TAR)
LACQUER DILUENTS
"MINERAL SPIRITS" RANGE 300°-400°F.
"SOLVENT NAPHTHAS"-AV BP NEAR 300°F
KAURI-BUTANOL SOLVENT POWER (1,2)
GARDNER-HOLDT TUBE LETTERS & CORRESPONDING POISES

FIGURE 6

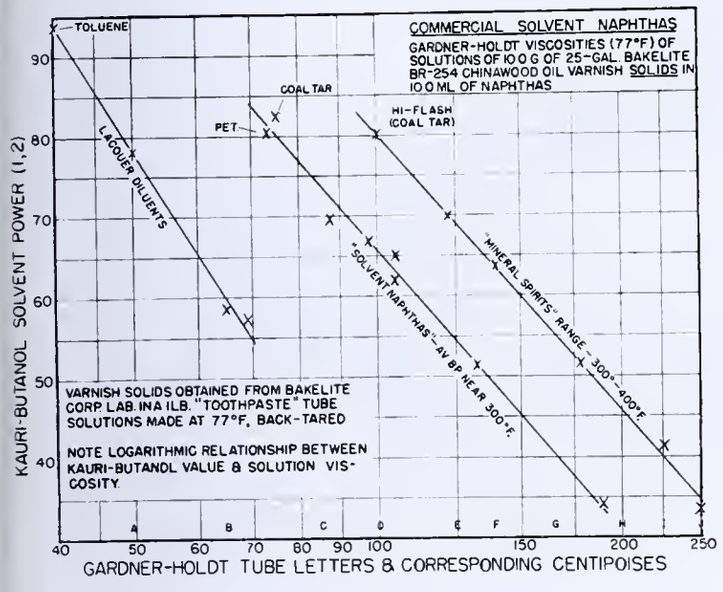
COMMERCIAL SOLVENT NAPHTHAS
GARDNER-HOLDT VISCOSITIES (77°F) OF SOLUTIONS OF 100 G OF 25-GAL. BAKELITE BR-254 CHINAWOOD OIL VARNISH SOLIDS IN 100 ML OF NAPHTHAS
TOLUENE
COAL TAR
PET.
HI-FLASH (COAL TAR)
LACQUER DILUENTS
"MINERAL SPIRITS" RANGE 300°-400°F.
"SOLVENT NAPHTHAS"-AV BP NEAR 300°F.
VARNISH SOLIDS OBTAINED FROM BAKELITE CORP. LAB. IN A I.B. "TOOTHPASTE" TUBE SOLUTIONS MADE AT 77°F. BACK-TARED
NOTE LOGARITHMIC RELATIONSHIP BETWEEN KAURI-BUTANOL VALUE & SOLUTION VISCOSITY
KAURI-BUTANOL SOLVENT POWER (1,2)
GARDNER-HOLDT TUBE LETTERS & CORRESPONDING CENTIPOISES

FIGURE 7

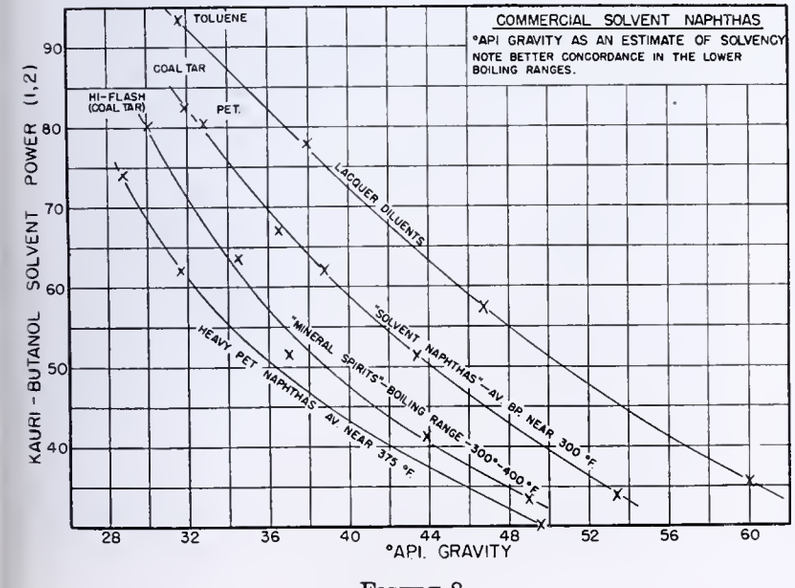

FIGURE 8

Composition

Marketers of petroleum solvent naphthas customarily offer a line wherein each member is designed to perform satisfactorily wherever a need exists for a product of its volatility or solvency, or both. Thus, in order to meet the most difficult requirements which may be encountered, most present-day petroleum solvent naphthas are more highly refined than is ordinarily necessary, and consequently contain less than a total of 1 per cent of unsaturated hydrocarbons and objectionable sulfur compounds. Solvency, therefore, can be readily estimated in terms of a naphtha's content of paraffin, naphthene, and aromatic hydrocarbons. Since the solvency characteristics of each group have been recorded (*1*, *2*, *27*, *30*), proximate analysis not only provides a convenient control check, but also facilitates the correct replacement of a naphtha—or pair of naphthas—for the one or pair already in use.

Volatility *vs.* Solvency

In the foregoing list, no mention has been made of the critical importance of volatility to the correct interpretation of any solvency test. Unfortunately, all too many discussions of solvency neglect this vital point. Purchasers interested in making replacements, or testing new offerings, are ordinarily concerned with products of similar volatilities, and are likely to overlook slight differences in boiling range, average boiling point, evaporation rate, or average molecular weight. Many such apparently slight differences, however, can well make one naphtha and break a substitute, if disregarded in connection with otherwise impartial evaluations of solvency (Figure 1). Particularly mystifying are the discrepancies between solvency test results and actual performance among commercial "mineral spirits"—300°/400° fractions, with average boiling points lying between 330° and 355° F. In both their higher and lower solvency classifications no rhyme or reason seems to exist, unless the appraiser studies his distillation points and realizes that no two have identical average boiling points or average molecular weights. (Average boiling point is frequently taken, as herein, as the arithmetic average of the 10, 20, 30, 70, 80, and 90 per cent A. S. T. M. distillation points, and is obviously a measure of average molecular weight for any given hydrocarbon series. The 99 per cent–paraffinic naphthas shown in Figure 1 are, respectively, the 25%/45%, 25%/50%, 25%/55%, and 25%/60% heart cuts of a wide-cut alkylate containing 0.7 per cent olefins.)

It is contended, and herein not originally (*19*, *37*, *39*), that any two accurate and precise methods of measuring solvency, or performance, can be used interchangeably in so far as they apply to petroleum naphthas of the same volatility or average molecular weight. Figures 3 to 13, inclusive, correlate only a few among the large number of possible combinations, but should prove convincing to one familiar with the experimental limitations of the several test methods. Kauri-butanol value has been chosen for comparisons in an effort to find whether this most popular quality test will serve (1) as a substitute for other laboratory solvency tests, and (2) as a safe prediction of the performance of a solvent naphtha of any given volatility. Figures 2 and 14 show the change with volatility of kauri-butanol value and mixed aniline point, respectively.

Attention is called to the excellent concordance between kauri-butanol solvency and actual performance in terms of synthetic resin solution tolerance for low solvency thinners (Figure 5), and viscosity of a popular synthetic resin–tung

oil spar varnish (Figure 7). The latter example illustrates the logarithmic relationship between solvency and solution viscosity for dispersions of the same character. Solutions of certain synthetics, however, do not follow this relationship throughout the entire solvency range of commercial solvent naphthas. Figure 6, dealing with a phenol-modified and oil-extended alkyd, indicates a change in the character of the dispersion with an increase in naphtha aromaticity. However, this not strictly logarithmic relationship with kauri-butanol solvency is nevertheless a smooth curve, and curvature is so gradual over any applicable solvency range that the function therein is essentially logarithmic. (Plotted viscosities range from 165 to 4600 centipoises.)

As a replacement for other laboratory solvency tests, kauri-butanol value is seen (Figures 3, 4, 8, 10–13) to provide a linear, or nearly linear, correlation of solvency for products of similar evaporation rates. Particularly replaceable, as straight-line functions, are such specification tests as mixed aniline point, specific dispersion, and per cent aromatics. Thus, bearing in mind that the laboratory requirements of kauri-butanol value comprise only 20 grams of a purchasable or easily made standard solution, one rough weighing, a simple titration, and no calculations—15 minutes of an inspector's time—whenever a need arises for a single solvency or performance test which covers the entire commercial naphtha solvency range and is applicable to products of any established volatility, kauri-butanol value seems to supply a ready answer.

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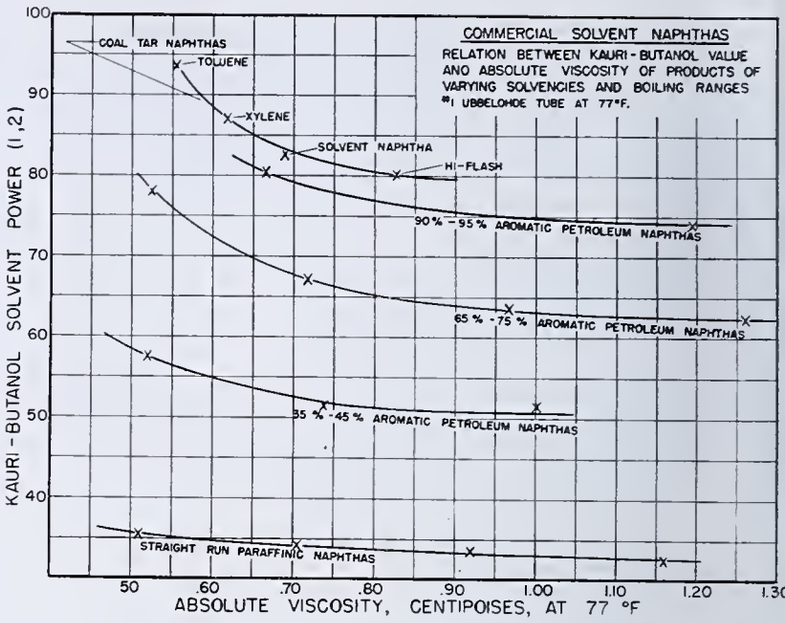
COMMERCIAL SOLVENT NAPHTHAS
RELATION BETWEEN KAURI-BUTANOL VALUE AND ABSOLUTE VISCOSITY OF PRODUCTS OF VARYING SOLVENCIES AND BOILING RANGES
IN I UBBELOHDE TUBE AT 77°F.
COAL TAR NAPHTHAS
TOLUENE
XYLENE
SOLVENT NAPHTHA
HI-FLASH
90% - 95% AROMATIC PETROLEUM NAPHTHAS
65% - 75% AROMATIC PETROLEUM NAPHTHAS
35% - 45% AROMATIC PETROLEUM NAPHTHAS
STRAIGHT RUN PARAFFINIC NAPHTHAS
KAURI-BUTANOL SOLVENT POWER (1,2)
ABSOLUTE VISCOSITY, CENTIPOISES, AT 77 °F

FIGURE 9

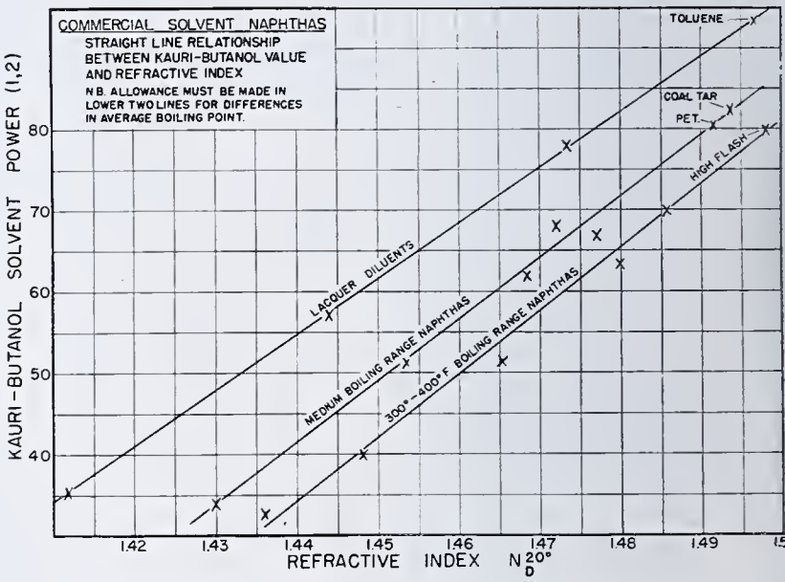
COMMERCIAL SOLVENT NAPHTHAS
STRAIGHT LINE RELATIONSHIP BETWEEN KAURI-BUTANOL VALUE AND REFRACTIVE INDEX
N.B. ALLOWANCE MUST BE MADE IN LOWER TWO LINES FOR DIFFERENCES IN AVERAGE BOILING POINT.
TOLUENE
COAL TAR PET.
HIGH FLASH
LACQUER DILUENTS
MEDIUM BOILING RANGE NAPHTHAS
300°-400° F BOILING RANGE NAPHTHAS
KAURI-BUTANOL SOLVENT POWER (1,2)
REFRACTIVE INDEX $N_D^{20°}$

FIGURE 10

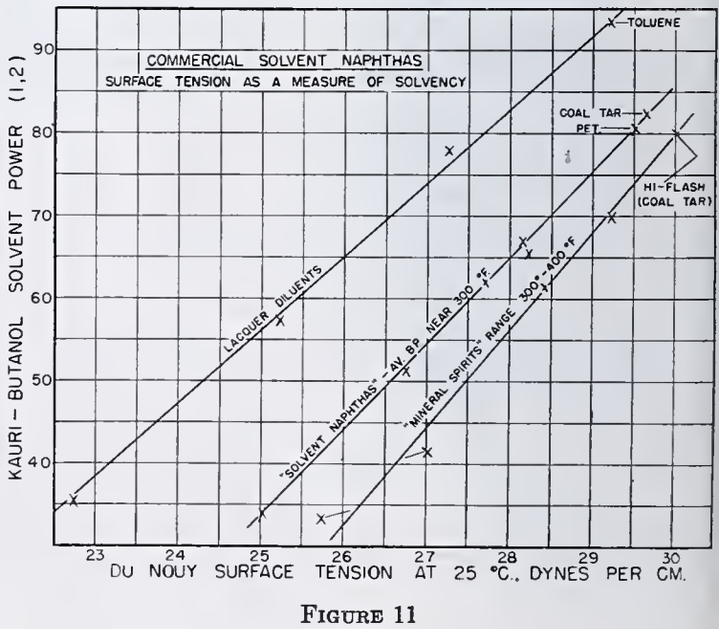
COMMERCIAL SOLVENT NAPHTHAS
SURFACE TENSION AS A MEASURE OF SOLVENCY
TOLUENE
COAL TAR PET.
HI-FLASH (COAL TAR)
LACQUER DILUENTS
"SOLVENT NAPHTHAS" - AV. B.P. NEAR 300 °F.
"MINERAL SPIRITS" RANGE - 300°-400 °F
KAURI-BUTANOL SOLVENT POWER (1,2)
DU NOUY SURFACE TENSION AT 25 °C., DYNES PER CM.

FIGURE 11

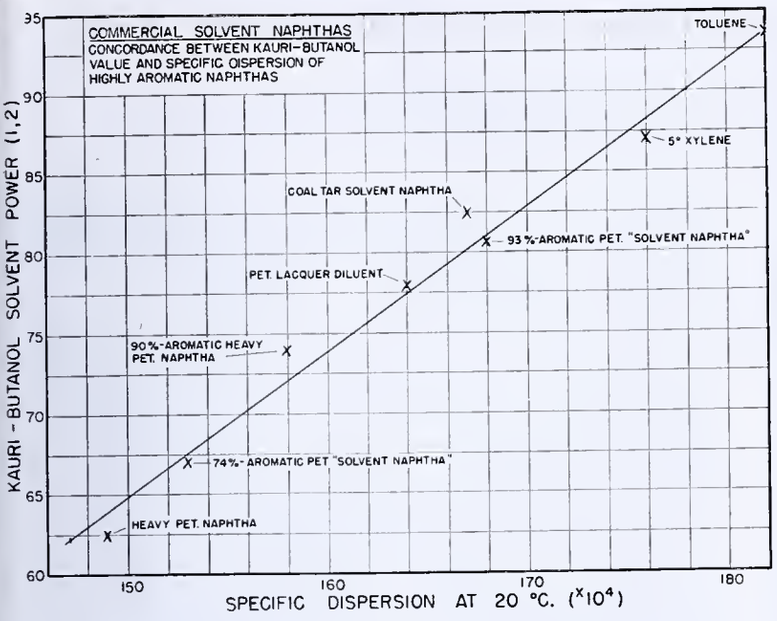
COMMERCIAL SOLVENT NAPHTHAS
CONCORDANCE BETWEEN KAURI-BUTANOL VALUE AND SPECIFIC DISPERSION OF HIGHLY AROMATIC NAPHTHAS
TOLUENE
5° XYLENE
COAL TAR SOLVENT NAPHTHA
93%-AROMATIC PET. "SOLVENT NAPHTHA"
PET. LACQUER DILUENT
90%-AROMATIC HEAVY PET. NAPHTHA
74%-AROMATIC PET "SOLVENT NAPHTHA"
HEAVY PET. NAPHTHA
KAURI-BUTANOL SOLVENT POWER (1,2)
SPECIFIC DISPERSION AT 20 °C. (X10⁴)

FIGURE 12

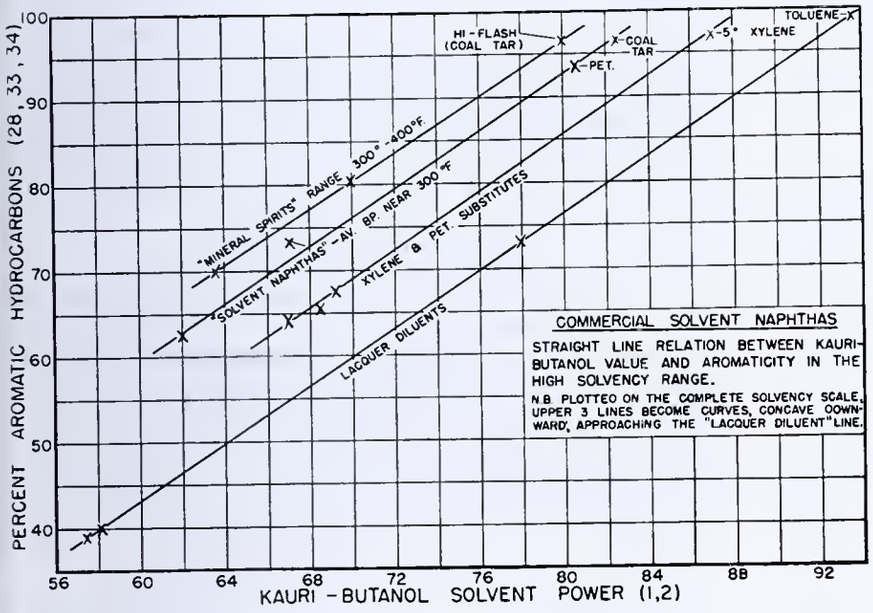
HI-FLASH (COAL TAR)
COAL TAR
PET.
5° XYLENE
TOLUENE
"MINERAL SPIRITS" RANGE 300°-400°F.
"SOLVENT NAPHTHAS"-AV. BP. NEAR 300°F
XYLENE & PET. SUBSTITUTES
LACQUER DILUENTS
COMMERCIAL SOLVENT NAPHTHAS
STRAIGHT LINE RELATION BETWEEN KAURI-BUTANOL VALUE AND AROMATICITY IN THE HIGH SOLVENCY RANGE.
N.B. PLOTTED ON THE COMPLETE SOLVENCY SCALE, UPPER 3 LINES BECOME CURVES, CONCAVE DOWNWARD, APPROACHING THE "LACQUER DILUENT" LINE.
PERCENT AROMATIC HYDROCARBONS (28, 33, 34)
KAURI-BUTANOL SOLVENT POWER (1,2)

FIGURE 13

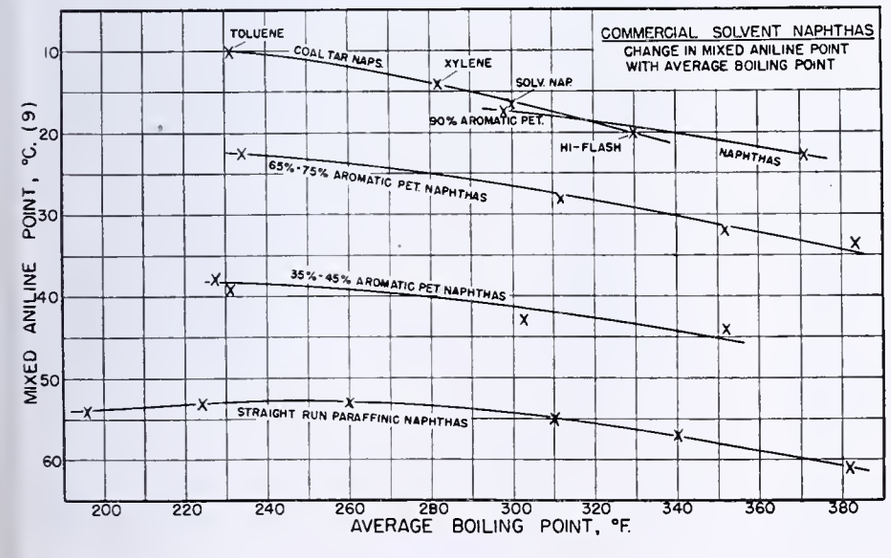

FIGURE 14

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Instrumental Methods of Chemical Analysis

FOLLOWING the precedent set by the October, 1940, ANALYTICAL EDITION, which dealt with American apparatus, instruments, and instrumentation, the October, 1941, issue will contain a systematized classification of instrumental methods of chemical analysis, covering the fundamental theory, description of equipment, and applications. Key references and the more important literature sources for each method will be included.

Readers of this journal can render invaluable aid to this undertaking and to their fellow chemists by supplying illustrations of actual installations with brief description or reference to any pertinent publications. Such examples will be of interest if they present results of high precision, ways of time-saving, handling large volume of work, or operation by relatively unskilled help, or are completely automatic.

Universities, research institutes, industrial laboratories, instrument companies, and governmental services, as well as individuals are earnestly requested to cooperate, so that the general treatment may be enhanced by illustrations of actual installations.

Information or questions should be addressed to Ralph H. Müller, Department of Chemistry, New York University, Washington Square East, New York, N. Y.

Evaluation and Performance of Turbine Oils

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Shell Oil Company, Inc., Wood River, Ill.

Progress in the design and construction of steam turbines has introduced new problems in lubrication and aggravated old ones. The importance of predicting the behavior in service of turbine oils has led to the development of suitable accelerated laboratory tests. Two essential requisites of a good turbine lubricant are the ability to protect steel surfaces against rusting and high stability toward oxidation. These tests are capable of revealing wide differences among turbine oils which meet conventional specifications. A discussion is presented of the chemical factors which influence the performance of oils in relation to the various methods employed by turbine operators to maintain an oil in serviceable condition.

IT IS becoming increasingly recognized that the performance of lubricating oils in modern steam turbines is but slightly if at all predictable from the conventional tests upon which specifications are commonly based. In general, these tests are able to exclude carelessly refined oils but make no distinctions as to the ultimate suitability of various well-refined products. Furthermore, progress in the design and construction of steam turbines has raised new problems in lubrication and aggravated old ones.

Since oils were employed in early steam turbines merely to provide lubrication, no special processing was necessary. With the advent of larger turbine installations, oil-circulating systems were introduced and several other functions were assigned to the oil. In addition to conventional hydrodynamic lubrication of the turbine shaft and thrust bearings and, in large units, also of couplings, the oil was required to act as a coolant. More recently, the same oil has been employed as a transfer medium to actuate the governor, and in geared units it lubricates high-speed gears. Development of larger and more efficient turbines operating at higher steam pressures and temperatures has further increased the demands on the lubricant.

Early turbine oils lacked stability toward oxidation and the products formed caused emulsification with water, foaming, and deposition of sludge (*5*). At first the improvement of turbine oils was chiefly directed toward reduction of their sludging tendency. It soon became apparent, however, that oil-soluble deterioration products (organic acids, etc.) were also harmful. Attention therefore began to be directed toward oxidation characteristics, to the neglect of other considerations such as ability to wet metal surfaces and thus to prevent rusting of turbine parts in the presence of water. The earlier-type turbine oils either contained certain components which promoted the formation of an adherent film of oil or soon produced similar compounds through oxidation. Improvement in sludging and emulsification characteristics necessitated by the more exacting requirements of the newer turbines was brought about largely by the application of modern refining methods. The desirable wetting agents responsible for the antirusting quality were, however, removed by these methods and the stable oils produced became inferior to less heavily refined or partly deteriorated oils with respect to rust-preventing characteristics.

In the presence of oils which provide no protective coating, steel surfaces are attacked by water with the formation of ferric oxide and black magnetic oxide. As rusting proceeds, these substances scale off and may be carried in suspension in the oil. The oxide particles sometimes score bearings, plug oil lines, and lead to faulty operation or even sticking of delicate governor parts. Difficulties due to rusting in the governor mechanism of new turbogenerators have in the past few years become prevalent, often causing severe economic loss (*1*).

It is the purpose of the present paper to advance methods for evaluating turbine oils, particularly in reference to stability and rust-prevention. This work has involved the development of laboratory tests for predicting these important characteristics, as well as a critical review of the older tests for properties. The usefulness of these performance tests in discriminating between turbine oils meeting ordinary specifications is demonstrated by application to a wide variety of such products. Finally, the chemical factors studied in the development of an accelerated test for the stability of turbine oils are considered in relation to their effect upon performance in service.

Testing of Turbine Oils

It is clear that two types of test are indispensable in both the production and application of modern turbine oils: (1) methods must be available for determining the actual properties of a lubricant in service so that progressive deterioration may be followed; (2) accelerated laboratory tests are required for predicting performance characteristics in a reasonable time (*36*). Standard testing procedures have been adopted where significant and certain new testing methods

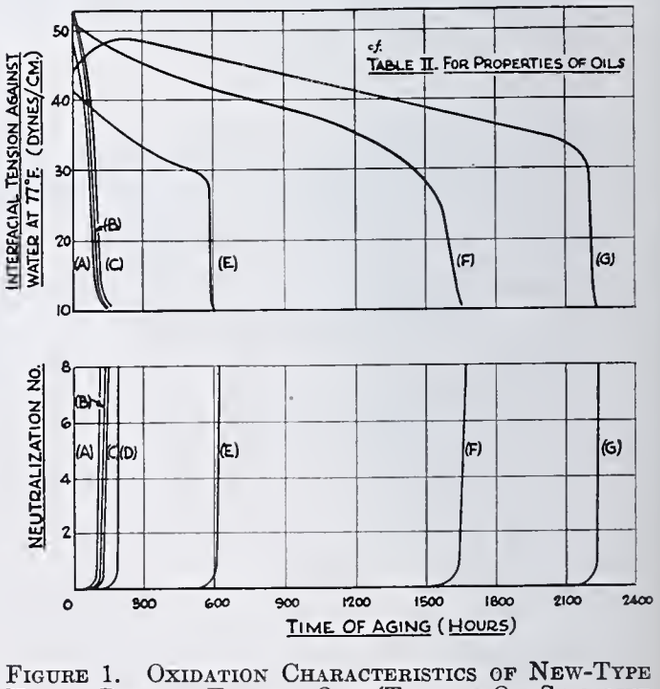

FIGURE 1. OXIDATION CHARACTERISTICS OF NEW-TYPE HIGHLY REFINED TURBINE OILS (TURBINE OIL STABILITY TEST)

have been devised in cooperation with turbine manufacturers and operators.

Tests for Instantaneous Properties. *Interfacial Tension against Water.* This property is a function of certain polar molecular groups present in an oil and bears a relation to its emulsifying tendencies. It may conveniently be measured by the pull-ring method employing the du Nöuy interfacial tensiometer. Cloudy oil samples should be filtered (or centrifuged) before testing to avoid contamination of the water phase in the measurement. Scale readings are converted to absolute values by means of a correction factor (*37*) which depends on the difference in density of the phases and on the dimensions of the ring.

New highly refined oils usually have high interfacial tensions (above 40 dynes per cm.) but formation of oxidation products (or contamination with used oil) sharply reduces the value. It is well known that polar molecules (soaps, acids, etc.) concentrate at the oil-water interface and orient themselves with the carboxyl groups toward the water phase; they can thus produce marked effects even when present in minute concentrations. This fact is used to great advantage because, in early stages of oxidation while mere traces of impurities are formed (values down to 30 dynes per cm.), interfacial tension is the most practical means for detecting them (Figure 1).

Interfacial tension measurements have been employed as a control in the refining of lubricating oil (*30*). They were also recommended to the first turbine oil committee of the A. S. T. M. about 20 years ago (*13*) for following the deterioration of turbine oils. Determinations were carried out by a drop-volume method using a modified Donnan pipet and aqueous phases of pure water and 0.01 *N* sodium hydroxide (*7*).

Neutralization Number. This is determined by a standard procedure (A. S. T. M. Designation D188-27T, Method A) and measures the acid content expressed in milligrams of equivalent potassium hydroxide per gram of oil. Since well-refined turbine oils have no measurable neutralization number (less than 0.05), free acids are a sign of oil deterioration or contamination. These substances are emulsifying agents and seriously affect the interfacial tension of the oil. When the interfacial tension drops below 30 dynes per cm., measurable quantities of acids are usually present. Some organic acids attack copper and iron and form oil-soluble soaps which, in addition to being promoters of emulsification, act as powerful oxidation catalysts.

Since various types of acids are formed on oxidation, depending on operating conditions and oil composition, a strict limit on neutralization number cannot be set. Highly refined oils should generally not exceed a neutralization number of 1.0 in service. At this point the interfacial tension of the oil has become extremely low (15 or less) and permanent emulsions often tend to form.

While interfacial tension and neutralization number are normally sufficient to characterize the condition of a highly refined turbine oil in service, the following tests can provide additional information:

Saponification Number (A. S. T. M. Designation D94-36). This value is expressed in the same units as the neutralization number but includes esters, soaps, and some potential acids (peroxides) in addition to free acids. The saponification number is thus always larger than the neutralization number (often as much as five to ten times as high) and embraces a large part of the oxidation products present in the oil. A sharp increase in saponification number is an indication of approaching oil breakdown and sludge formation. The saponification number of sludge is very high.

Viscosity Increase. An increase of the oil viscosity in service is due to an accumulation of oil-soluble oxidation products and is a forerunner of sludge formation.

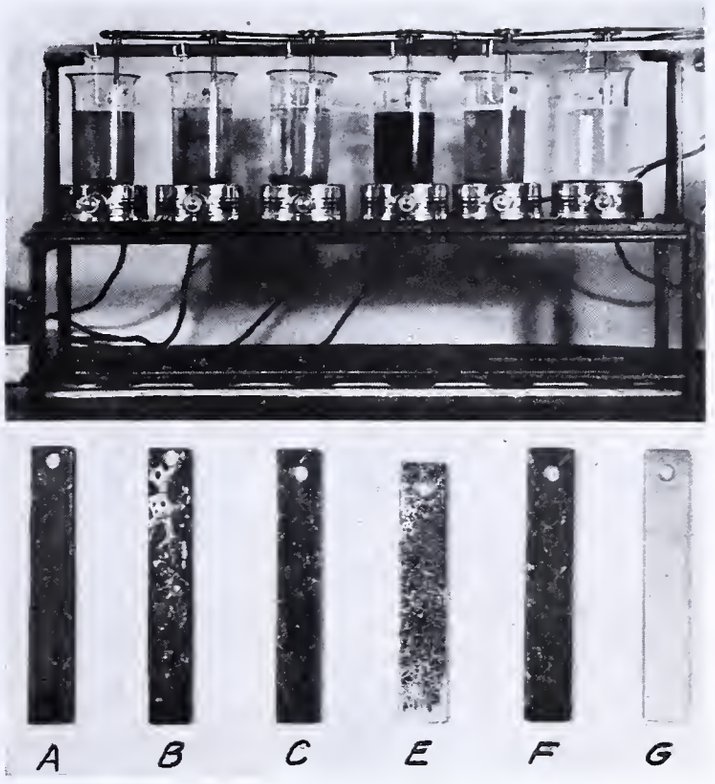

Figure 2. Rusting Tests on Six New-Type Highly Refined Oils after 48 Hours

Above. Assembly
Below. Specimens

Sludge. This determination has lost much of its significance with the advent of modern nonsludging turbine oils. The oxidation products in such oils remain in solution long after the oil has become unfit for service. However, permanent water-oil emulsions, which usually contain some metal soaps as stabilizers, often clog filters and appear as sludge. Sludge deposits in neglected turbine installations present a serious problem because they not only interfere with lubrication but also drastically reduce the life of subsequent charges of oil. The ash content of sludge consists mainly of iron oxide, often with a trace of copper (*3*, *27*).

Appearance. Badly deteriorated oils are usually very dark colored. Occasionally, however, an oil shows a persistent haze or a dark-brown opaque appearance without having a low interfacial tension, high neutralization number, or pungent odor. This is due to traces of iron soaps and colloidal hydrated iron oxide from rust and sludge deposits in the oil system. Such contamination usually reduces the life of the oil. Rust may also form in new turbines at an early period of operation if the oil used has no antirusting properties. In each case a trace analysis will show the presence of iron.

Dithizone Test. Most oil coolers are constructed of copper or bronze tubes, and copper lines are often present elsewhere in the oiling systems. Some organic acids formed on deterioration of an oil attack copper, forming oil-soluble soaps which are powerful catalysts for oxidation. Since the presence of these soaps is always an indication of actual or impending breakdown of an oil, their detection is of interest. This may be accomplished by the dithizone test as follows: A solution of diphenylthiocarbazone (dithizone) in carbon tetrachloride (5 mg. per 100 ml.) is added to the oil under investigation. Peroxides, which interfere, may be removed by a preliminary reduction with sulfur dioxide. If dissolved copper is present, the green dye turns red. Concentrations of the order of one part per million can be detected. This reagent was first applied by Fischer (*11*, *12*) and the procedure was later adapted to the determination directly in oil solution by Waring (*32*).

Steam Emulsion Number (A. S. T. M. Designation D157-36). This is generally recognized as the best method now available for determining the tendency of oil to separate water. However, interpretation is difficult, since no account is taken of the water remaining in the oil phase at the completion of the test. Furthermore, the relation between steam emulsion number and state of deterioration differs markedly for oils of large and small molecular size. The "demulsibility test" is of less value because it involves the wrong type of emulsion (oil-in-water).

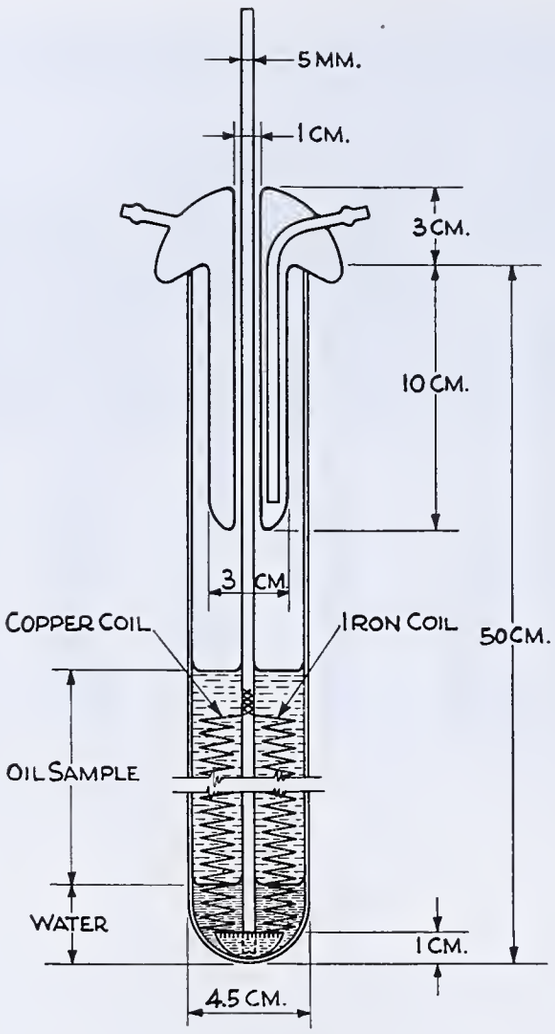

FIGURE 3. OXIDATION CELL FOR TURBINE OIL STABILITY TEST

ACCELERATED TESTS FOR PREDICTING PERFORMANCE CHARACTERISTICS. *Turbine Oil Rusting Test.* A clear distinction should be made between several kinds of corrosion which may occur in turbines. One type, which has been experienced for many years in the upper parts of oil reservoirs, etc., is caused by the action of water vapor and volatile acids from oxidized oil. Since the metal surfaces involved are not normally covered by oil, this is not essentially a problem in lubrication but can probably best be solved by application of a protective coating.

A second type of corrosion is the attack of metal surfaces, which come in contact with the oil, by organic acids formed through oxidation. This problem can be eliminated by using an oil of high stability.

In the present paper primary consideration is given to a type of attack which often occurs after only a short period of operation with new oils free of acid. This is purely a rusting phenomenon caused by droplets of liquid water coming in contact with steel surfaces which have presumably been covered by the lubricant and displacing it. An oil will protect against this more serious action only if it wets steel surfaces preferentially as compared with water.

The importance of the antirusting quality in turbine oil has become recognized. A test devised three years ago by Kuebler (*21*) in the laboratories of one of the major turbine builders employs a highly polished steel specimen suspended in the oil to be tested which is then stirred for a time to ensure wetting of the specimen. One per cent by volume of distilled water is added and stirring continued 8 hours a day for 5 days at room temperature.

In modifying the test in these laboratories to comply more nearly with conditions met in operation, the testing temperature is raised to 75° C., 10 per cent of water is added instead of 1 per cent, and stirring is carried on continuously for 48 hours. Under these more severe conditions rusting naturally proceeds rapidly in any oil which allows rust formation at room temperature. However, the converse is not true, for an oil which is effective at room temperature does not necessarily protect steel surfaces at 75° C. The more discriminating test has given results in better agreement with service data.

The general arrangement of the test apparatus is shown in Figure 2. The oil samples are contained in 600-ml. heavy-walled Pyrex beakers which are mounted on thermostatically controlled hot plates. Vigorous stirring is provided by all-glass stirrers. The test specimens are low-carbon cold-rolled steel with a hole at one end to allow suspension by means of an enameled or lacquered wire. They are ground, polished, and cleaned before use and must not be touched with the fingers; great care must be exercised in preparing the strips to ensure reproducible results. The specimen should not touch the sides of the beaker because a dead space will form in which water remains stationary, causing erratic results. Water is added during the test to replace that lost by evaporation. At the conclusion of the test the specimens are washed with naphtha and acetone and examined visually. Extent of rusting is expressed in per cent of surface rusted. Photographs of specimens from tests (Figure 2) show the wide variation in antirusting characteristics of several modern-type turbine oils.

Turbine Oil Stability Test. Turbine oils are expected to withstand continuous operation at moderately elevated temperatures in the presence of air, water, and metals without forming sludge or emulsions. Their service conditions are essentially different from those of automotive lubricants and, hence, efforts to evaluate turbine oils by commonly accepted oil-testing methods carried out at excessive temperatures were singularly unsuccessful. One of the first tests adapted specifically for turbine oils was that of Funk (*14*, *15*). This test was later modified and other tests were devised (*6*, *10*, *23*).

The turbine oil stability test, developed in these laboratories for predicting the useful life expectancy of turbine oils, combines several features of earlier tests (*10*, *20*, *23*, *26*, *29*). Samples of oil are aged under accelerated conditions which do not deviate qualitatively from those met in practice and with the provision that all factors influencing rate of deterioration are maintained constant.

A 300-ml. sample of the oil under investigation is placed in a large test tube, 60 ml. (20 per cent by volume) of distilled water are added, and metal catalysts are introduced (a coil of iron and a coil of copper wire joined together). Pure iron wire (analytical grade) is employed, whereas copper wire ordinarily manufactured for electrical purposes is satisfactory. Both metal surfaces are carefully cleaned before using. Enough wire is taken to give 0.5 sq. cm. of copper surface and 0.5 sq. cm. of iron surface exposed per ml. of oil. Pure oxygen is supplied to the oil at a rate of 3 liters per hour through a glass tube terminating in a fritted-glass plate which disperses the gas into fine bubbles, ensuring intimate contact with the liquid and vigorous agitation. The oxygen escapes through a reflux condenser which retains water and volatile oxidation products to a considerable extent.

The oxidation cell (Figure 3) is immersed in a thermostated bath maintained at 100° ± 1° C. and the temperature of the sample comes to equilibrium at some value a few degrees lower because heat is continually lost through evaporation of water. In the tests carried out in these laboratories, the temperature was 95° ± 1° C.; it may deviate somewhat from this figure in other apparatus if the rate of heat transfer is different. The water which is lost slowly throughout the test is replaced occasionally, so as to maintain its level at least 3 cm. above the sintered plate. Test tubes, thermostat, and oxygen-flowmeters are, for convenience, similar to those employed in the Indiana oxidation test (*2*).

Small portions of the oil are withdrawn for interfacial tension and neutralization number measurements at intervals, as determined by appearance or the emanation of acidic odors, and the results are plotted as a function of time (Figures 1 and 4). Parts of the copper and iron coils are also removed, to maintain a constant ratio of metal surface to oil volume; the frequency of sam-

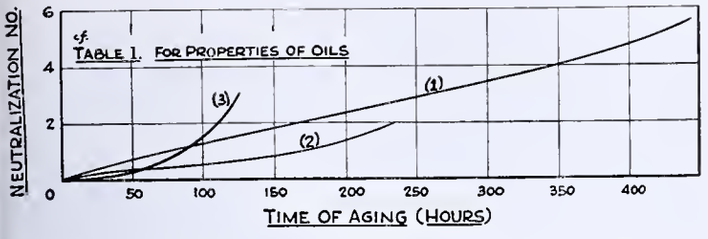

FIGURE 4. OXIDATION CHARACTERISTICS OF EARLY-TYPE LIGHTLY REFINED TURBINE OILS (TURBINE OIL STABILITY TEST)

pling then has no appreciable effect on the results. It can be seen that interfacial tension is more significant during early stages of oxidation, whereas neutralization number is more indicative when appreciable deterioration has occurred. Appearance and other tests previously described may yield significant information.

With careful manipulation the life of an oil, taken as the time to reach a neutralization number of 1.0, is reproducible within a ±5 per cent range. The test correlates well with practice, inasmuch as oils are rated in the same relative order as by their known service records. Because of the wide variation in operating conditions to which oils are subjected, a hard and fast prediction of their life expectancy in a given turbine cannot be made on the basis of any laboratory test. For example, it has been found that the ratio of the useful life of a certain oil in service to its life in the turbine oil stability test has ranged from 200:1 to 5:1.

Factors Influencing Oil Deterioration in Laboratory Tests

Several important variables have been investigated in the laboratory in establishing the above conditions for accelerated aging.

OXYGEN FLOW RATE. It is obviously desirable to maintain the flow rate at so high a value that the oil is kept saturated with oxygen at all times; the reaction velocity will then be insensitive to variations in flow above that rate. Data obtained at several rates (all other variables being held constant) are given in Figure 5, *A*. It was found that 3 liters per hour are sufficient for saturation but 1 liter per hour seemed to be somewhat inadequate. Excessively high flow rates should not be employed, so as to avoid the rapid elimination of volatile oxidation products (see Volatile Oxidation Products, below).

TEMPERATURE. Although no study of temperature coefficients has been made, reaction velocity is doubtless strongly dependent on this variable and precise control is therefore essential. The lower the temperature, the more time is required for testing. The temperature adopted is as high as is compatible with the presence of liquid water at atmospheric pressure.

METAL CATALYSIS. The general catalytic effect of metals on oil oxidation has been known for some time (*33*), but there has been confusion as to the difference in action of the various metals (*8*, *9*, *20*, *28*, *33*, *34*, *35*). Recently copper and iron have received special attention (*10*, *23*, *29*). Since both these metals are present in turbine lubricating systems, they were included in the laboratory stability test. The rate curves given in Figure 5, *C*1, demonstrate clearly the accelerating action of both metals, singly and in combination, in the presence of water. Although the activity of iron is less than that of copper per unit area, catalysis by iron may be the more important because of its greater extent of surface in a turbine. Since the reaction velocity varies with surface area exposed per unit quantity of oil (Figure 5, *C*2), the importance of keeping this ratio constant is evident.

The two metals together are less active than copper alone (Figure 5, *C*1) probably because iron, which stands higher in the electromotive series, hinders the solution of copper, which is the more active catalytically. Different results were obtained when copper and iron coils were connected together and when they were separated (Figure 5, *C*3). The former arrangement was adopted for the test so as to parallel turbine construction; good electrical contact must be maintained at all times in order to avoid variable galvanic effects which may lead to erratic results.

Since the use of tin-plated copper tubes has been proposed for turbine oil coolers, a single experiment was performed with

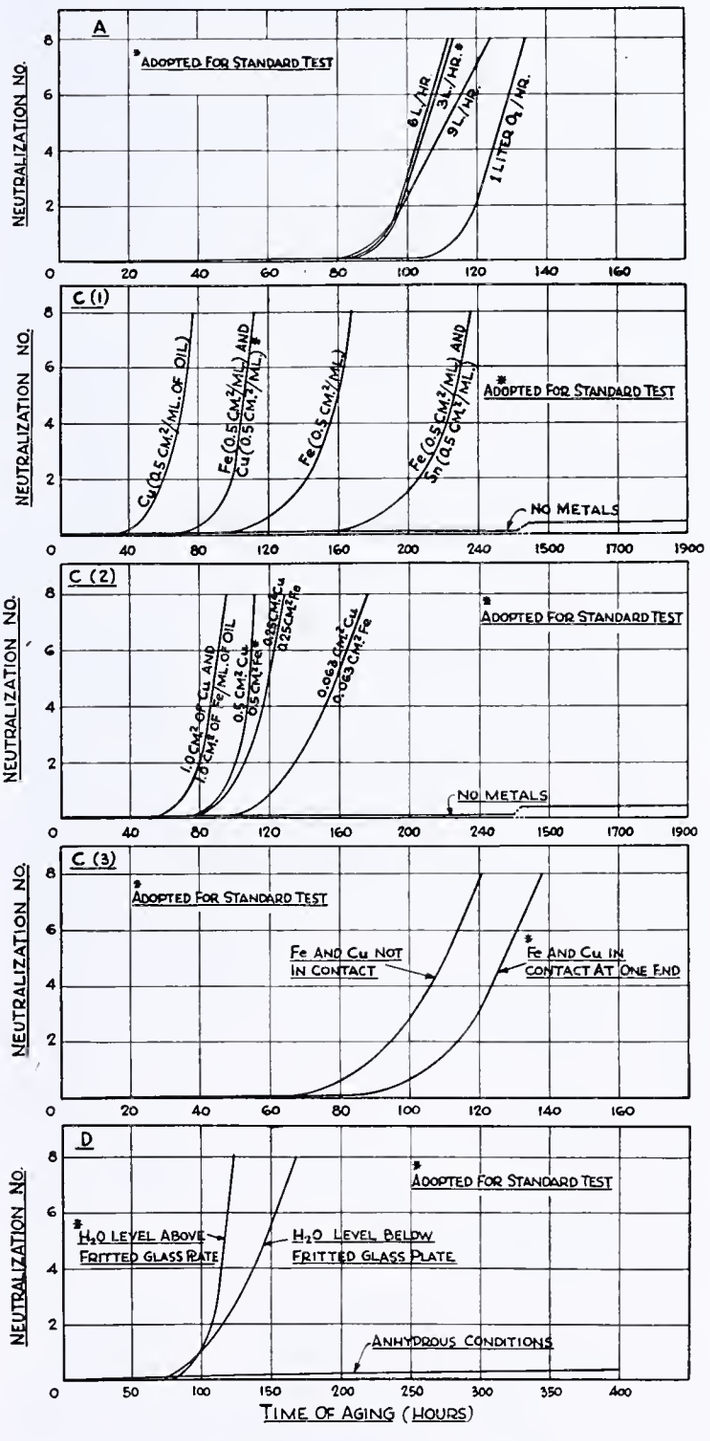

FIGURE 5. FACTORS INFLUENCING OIL DETERIORATION IN TURBINE OIL STABILITY TEST

A. Oxygen flow rate
C. Metal catalysis: (1) kind of metal, (2) ratio of metal surfaces to oil volume, (3) galvanic effects
D. Effect of water

TABLE I. PROPERTIES OF EARLY-TYPE LIGHTLY REFINED TURBINE OILS

Oil designation	1	2	3
Gravity, °A. P. I.	26.6	28.6	24.9
Color, N. P. A.	3+	5+	6+
Pour point, ° F.	+15	+25	+30
Flash point, ° F. (O. C.)	390	395	415
Fire point, ° F. (O. C.)	450	455	475
Viscosity at 100° F., S. U. seconds	177	281	480
Viscosity at 210° F., S. U. seconds	40	52	59.5
Viscosity index, Dean and Davis	62	104	82
Neutralization number	0.05	0.05	<0.05
Saponification number	1.6	0.5	0.4
Interfacial tension against water at 77° F., dynes per cm.	37	34	41.5
Steam emulsion number	180	560	330
Specific dispersion (*31*)	125.5	123	132

TABLE II. PROPERTIES OF NEW-TYPE HIGHLY REFINED TURBINE OILS

Oil designation	A	B	C	D	E	F	G
Gravity, °A. P. I.	30.4	31.6	29.6	33.6	31.1	32.8	32.1
Color, N. P. A.	−1	−1	−1	1+	−1	1½	−1
Pour point, ° F.	+20	0	+5	+5	+5	−5	+5
Flash point, ° F. (O. C.)	375	390	385	410	370	395	385
Fire point, ° F. (O. C.)	435	450	445	470	420	460	445
Viscosity at 100° F., S. U. seconds	146	156	156	156	157	153	156
Viscosity at 210° F., S. U. seconds	42.7	44.0	42.8	44.7	43.3	44.0	43.5
Viscosity index, Dean and Davis	95	107	81	118	91	111	97
Neutralization number	<0.03	<0.05	<0.03	<0.05	0.03	<0.03	<0.05
Saponification number	0.08	0.20	0.16	0.06	0.23	0.08	0.10
Interfacial tension against water at 77° F., dynes per cm.	48.5	52.5	52.5	43	41.5	51	44.5
Steam emulsion number	30	30	30	45	60	30	60
Specific dispersion (*31*)	108	105.5	110	100	97	106.5	102.5

tin and iron in place of iron and copper. As shown in Figure 5, *C*1, an oil life was obtained longer even than that observed in the presence of iron alone. It thus appears that metallic tin actually exerts a stabilizing effect. A similar action on the part of tin compounds has also been noted (*24*).

WATER. Water is usually found in turbine lubrication systems, originating from leaks in sealing glands and infrequently in coolers. The effect of water was therefore investigated in developing the stability test. Evidently, water alone is not a powerful catalyst, if at all, since reaction is very slow in its presence when metals are absent (Figure 5, *C*1). On the other hand, water, copper, and iron together accelerate oil oxidation much more than do the metals alone (Figure 5, *D*). It may, therefore, be inferred that water is a promoter for the action of metals, perhaps facilitating their solution in oil as soaps which then function by a mechanism of homogeneous catalysis. A single experiment performed with the water level maintained below the sintered plate gave the same oil life as that observed under standard testing conditions, but the final rate of deterioration (during the "break") was considerably lower. Apparently, the degree of agitation of both water and oil phases has an effect during later stages of oxidation.

CONTAMINATION WITH USED OIL. It is well known that the oxidation of highly refined oils is autocatalytic—i. e., the reaction velocity increases with time owing to catalysis by substances produced during the course of the reaction—e. g., peroxides and acids. This effect is especially pronounced when metals are present, since the acids then form copper and iron soaps which are extremely powerful pro-oxidants. In Figure 6 are presented data for the oxidation of blends of turbine oil with used oil from a turbine. Since the addition of only 10 per cent of a mildly deteriorated oil reduced the oil life by as much as 75 per cent, the accelerating action of oxidation products is clearly demonstrated. Furthermore, even traces of oxidized material have a perceptible effect on reaction velocity. It follows that scrupulous care must be taken in cleaning apparatus before the start of a run in order to ensure accurate and reproducible results.

VOLATILE OXIDATION PRODUCTS. The complex reactions occurring in oil oxidation inevitably lead to the formation of acids of various molecular sizes. It would be expected that those of low molecular weight would be the most powerful pro-oxidants, since they are generally the strongest acids and attack metals most readily to form catalytically active soaps. The loss of these low-boiling acids by volatilization during the turbine oil stability test thus becomes a significant rate-determining factor. A few experiments performed in apparatus which provides for complete recirculation in a closed system have actually given considerably higher reaction velocities than other measurements in which the lightest acids were continuously swept out of the oil by the stream of oxygen. Accordingly, reflux condensers have been adopted for the test to retain as much of the volatile acids (and water) as is practicable without unduly complicated equipment.

Evaluation of Turbine Oils

Properties of three early-type and several modern turbine oils are presented in Tables I and II. The former are probably distillate cuts from unextracted lubricating oil stock; the latter appear to be well-treated neutrals.

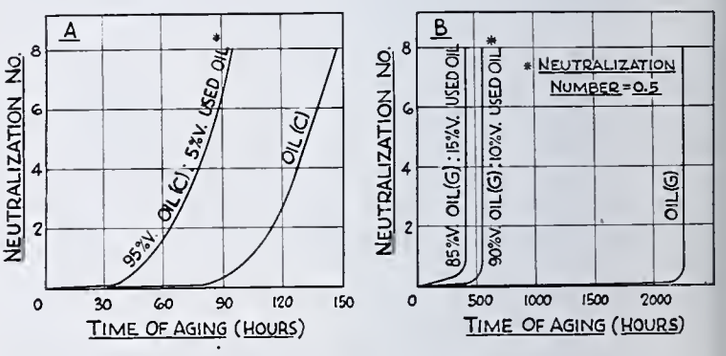

FIGURE 6. DELETERIOUS EFFECT OF USED OIL ON STABILITY OF TURBINE OILS (TURBINE OIL STABILITY TEST)

A. Oil of ordinary inherent stability
B. Oil of high inherent stability

As shown in Figures 1 and 4, the difference in composition of the two types of oil results in widely different oxidation characteristics. The modern oils remain in serviceable condition throughout an initial induction period during which deterioration is very slow, whereas the lightly refined ones react at moderate velocity from the start of the run, with early foaming, emulsification, and sludge formation. It is nevertheless significant that the various highly refined oils show a twentyfold variation in resistance toward oxidation in spite of their similarity in other properties. Apparently the less stable oils contain only those naturally occurring antioxidants which are not removed in the course of refining; the more stable ones doubtless contain added inhibitors.

In contrast to the superiority of highly refined products with respect to oxidation stability, lightly refined oils are often more satisfactory in regard to rust-preventing characteristics. A picture of the turbine oil rusting test is given in Figure 2; the specimen for oil D is not shown but resembles that for C. It is evident that modern-type oils may vary from zero to complete protection against rusting.

Factors Influencing Oil Deterioration in Service

The influence of several variables on oil deterioration in the turbine oil stability test has been discussed above. Since these factors control the performance of a lubricant in service, it is important that they be considered in establishing turbine-operating conditions so as to ensure the maximum useful life of an oil.

Aeration and Venting. During rotation of the turbine shaft a partial vacuum is created within the upper part of the bearing by an action like that of a rotary vacuum pump (*22*). Since the oxygen which is dissolved in the oil may become involved in oxidation reactions, provision should be made to eliminate it as quickly as possible. This is usually accomplished by venting the oil reservoir directly to the atmosphere or by applying gentle suction.

The vented air is often saturated with water vapor and, in cases where the turbine oil has deteriorated, also with volatile acidic oxidation products. These vapors, unless quickly released, not only accelerate the deterioration of the oil but also cause corrosion and promote rusting by water particularly in the vapor space of oil tanks, bearing housings, and gear cases. The oil vapors escaping through the vents, if condensed, should not be returned to the system under any circumstances. Dantsizen (*6*) has shown that aliphatic acids ranging from formic to butyric are active in promoting rusting.

Temperature. Like most chemical reactions, oil oxidation is sharply accelerated by a rise in temperature. The best turbine oil maintained at approximately 95° C. (as in the turbine oil stability test) lasts several months, but if held at 150° C. (under oxygen pressure in the presence of water and catalytic metals) the life becomes a matter of hours. Lowering the operating temperature is therefore an effective way to increase the life of an oil in service. Since in modern turbines steam temperatures are high and the units are being built more and more compact, proper cooling is important.

Settling tanks which are inserted in turbine-lubricating systems to permit the separation of water, etc., function best when the oil is kept warm; these tanks are therefore usually located ahead of the oil coolers. It is, however, advisable not to exceed a temperature of 55° C. in the oil settlers, so as to avoid unnecessary oil deterioration. The practice of heating the oil in the settlers with built-in electric heaters or high-pressure steam coils to break an emulsion is decidedly harmful in view of the high local temperatures.

Metal Catalysis. Since catalysis by copper and iron is a function of the ratio of metal surface to oil volume, the rate of deterioration can be materially reduced by increasing the oil capacity of the lubricating system.

The replacement of catalytic metals (especially copper) by aluminum has been suggested (*20*). While some progress in this line is possible, there are several limitations to be considered, such as the cost, tensile properties, and the susceptibility of aluminum to attack by water (particularly, sea water) in the coolers. The use of tin-plated oil lines has been proposed and might prove to be advantageous (*18*, *19*). Certain alloys might also be applicable. In any case the problem of catalysis by iron and copper remains serious, owing to the large number of existing installations containing both these metals.

Water. Water is a promoter for the catalytic action of metals on oil deterioration and may cause emulsification and rust formation. It is thus highly desirable that turbine installations have effective sealing glands which prevent the entrance of steam or water into the oil system. Furthermore, if water suddenly gains access to a highly deteriorated dry oil containing large amounts of oil-soluble oxidation products, emulsification and a sudden precipitation of sludge may occur.

In cases where the exclusion of water is impractical, it may be removed continuously by such methods as filtering, gravity-settling, or centrifuging. Filtration through clay has an adverse effect on modern turbine oils of high stability but cotton bags give excellent results. The use of a large oil volume permits a reduction in the rate of oil circulation and affords a better opportunity for the water to settle out. Centrifuging greatly accelerates the separation of suspended water, sludge, and solid particles—that is, all those impurities which if given a chance would settle out spontaneously—even in lubricating systems of relatively low capacity. However, caution should be exerted that the oil capacity is not reduced to extreme, as this may indirectly contribute to oil breakdown (see Metal Catalysis, above).

Used turbine oil is sometimes washed continuously with hot water to remove extractable oxidation products (acids); the water is then separated by centrifuging or filtration (*16*, *17*, *25*). This process can best be employed if water has already entered a system containing an appreciably deteriorated oil.

Contamination with Used Oil. Most cases of rusting known to the authors have occurred in new installations. Older units normally gave no trouble because some used oil from the previous fill, containing oxidation products capable of protecting steel, remained in the system after the conventional cleaning. This procedure usually consisted of manual scraping and wiping, steaming, and flushing with new oil. Accordingly, some turbine manufacturers and operators have turned to the addition of used oil to new oil fills. Although some protection against rusting has been obtained, this practice should be avoided since it greatly reduces oil life (Figure 6). Furthermore, very thorough cleaning of contaminated systems is necessary if the full life expectancy of an oil is to be realized. In three cases which exemplify this point a fresh highly stable oil of modern type was used in a new unit, in an older unit cleaned by conventional methods, and in one which was simply drained.

1. A new 35,000-kw. turbogenerator was charged with 15,140 liters (4000 gallons) of oil. The interfacial tension, originally 51 dynes per cm., dropped to 42 in 2000 hours and the unit has subsequently operated for 13,000 hours without measurable change in interfacial tension. The initial drop is attributed to contamination by break-in oil employed at the factory. No operating difficulties due to the lubricating oil have been noted.
2. An 8000-kw. turbogenerator cleaned in the conventional manner was charged with 1892.5 liters (500 gallons) of oil. The interfacial tension, originally 52 dynes per cm., fell to 37 within 24 hours and to 32 in 2000 hours. Although the unit has subsequently operated for 12,000 hours without further change in interfacial tension, emulsions tend to form, owing to contamination by oxidation products from the previous fill.
3. Two 785-horsepower turboblowers possessing a common oil system were charged with 3028 liters (800 gallons) of fresh turbine oil. No effort was made to clean the system after the old oil was drained. In only 1920 hours of operation the interfacial tension of the new oil had dropped from 48 to 23 dynes per cm., a measurable neutralization number and a hazy appearance had developed, and there were all the indications of an impending breakdown. In the turbine oil stability test the same oil had a lifetime of 2700 hours. Although these units operate under rather adverse conditions (bearing outlet temperature, 82.2° C., 180° F.), a much longer useful life would doubtless have been obtained were it not for the sludge left in the system from earlier operation.

It has been a custom in the past to remove periodically some of the used oil in a turbine and replace it with fresh oil. This practice, known as "sweetening", originated as a step to remove sludge. Since modern turbine oils do not form sludge during their useful life, this precaution is unnecessary. Any benefit is only temporary and the rate of oil deterioration is not reduced proportionally (*4*).

It is recommended for most efficient and economical operation that turbines be thoroughly cleaned with a suitable solvent before new lubricant is charged; modern-type oils containing added inhibitors should then function for years without appreciable deterioration. However, since the cleaning procedure removes contaminants which protect steel surfaces, rusting may be expected unless the lubricant employed affords protection.

Among numerous examples of the successful cleaning of neglected industrial and marine turbines, one in particular may be

noted. A small geared turbogenerator caused the breakdown of former oil fills in a few hundred hours of operation. After thorough application of a special cleaner, the interfacial tension of the new oil charge decreased by only 0.5 dyne per cm. in 24 hours and has remained practically unchanged during several thousand hours of subsequent operation.

Acknowledgment

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Ring Method for the Determination of Interfacial Tension

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THE ring method for the determination of surface and interfacial tension has come into common use during the past decade or so. Interfacial tension may be used in studying small changes in concentration of impurities during the refining of petroleum (*5*, *7*, *8*) that are difficult to detect by other means. It is likewise useful in following the deterioration of hydrocarbon oil, either in accelerated stability tests or in actual service (*5*, *6*, *9*, *10*). In applications of this nature the ring method is usually chosen on account of its simplicity.

Harkins and Jordan (*4*) have pointed out that values obtained by the ring method according to the simple equation $S = \frac{P'}{4\pi R}$ may be in error by as much as 30 per cent, or even more. In this equation S is the surface or interfacial tension in dynes per centimeter, P' is the maximum pull on the ring in dynes, and R is the radius of the ring in centimeters. These investigators have worked out, upon an empirical basis, factors which can be used to correct such values. These correction factors depend upon the density of the liquid or liquids, the maximum pull on the ring, and the dimensions of the ring. Freud and Freud (*2*) have substantiated this work from a fundamental standpoint.

Harkins and Jordan have prepared tables of their factors which are conveniently used for surface tension work. However, for interfacial tension measurements, the tables are inadequate except for the cases of very low scale readings and/or very great differences between the densities of the two phases. To handle the cases most generally met in the measurement of interfacial tension at hydrocarbon-water interfaces, it has been necessary to extend the data of Harkins and Jordan considerably.

The method employed consisted of a mathematical analysis of the tabulated data to ascertain whether they obeyed any "natural" mathematical relation which might constitute a reliable basis for extrapolation. It has been found that, for a given ring, the correction factor is determined by the relation:

$$(F - a)^2 = \frac{(4b}{(\pi^2} \cdot \frac{1)}{R^2)} \times \frac{P}{D - d} + C \qquad (1)$$

Table I. Agreement between Data of Harkins and Jordan and Calculated from Equation 1

R/r	$P/(D-d)$	F (H and J)	F (Equation 1)	R/r	$P/(D-d)$	F (H and J)	F (Equation 1)
30	105.36	1.012	1.016	50	105.36	1.054	1.053
	79.017	0.967	0.972		79.017	1.013	1.014
	52.678	0.921	0.918		52.678	0.970	0.969
40	105.36	1.038	1.040	60	79.017	1.022	1.023
	79.017	0.996	0.999		63.213	0.998	0.998
	52.678	0.950	0.951		52.678	0.981	0.980

where $F = \frac{S}{P}$ = correction factor

S = interfacial or surface tension, dynes per cm.
P = maximum pull on ring, dynes per cm.
D and d = densities of the lower and upper phases, respectively (liquid-air, water-oil), etc.

and for the case of a given ring the following quantities are constant:

R = the radius of the ring
C = a constant, which depends upon the ratio r/R, where r = radius of the wire of the ring, in the following manner:

$$C = 0.04534 - 1.679\,\frac{r}{R} \qquad (2)$$

and $a = 0.7250$ and $b = 0.0009075$ are universal constants for all rings.

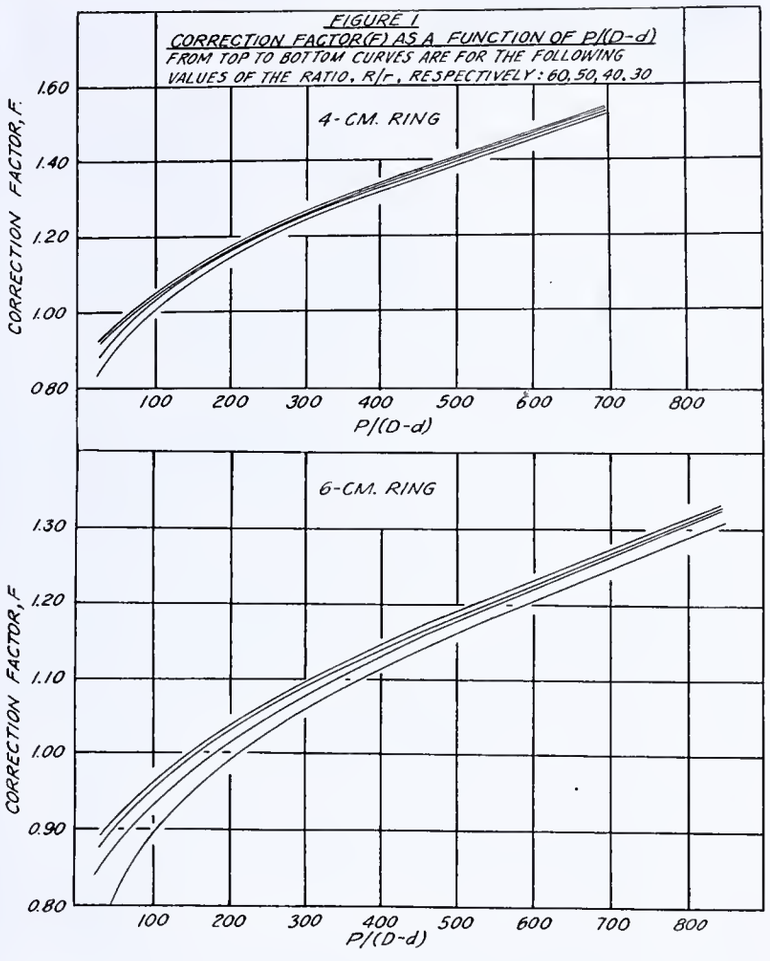
It is apparent from Equation 1 that the dependence of F upon the quantity $P/(D-d)$ obeys a parabolic law for any given ring. The ability of Equations 1 and 2 to evaluate the data of Harkins and Jordan is illustrated in Table I. This satisfactory agreement in conjunction with the simple and standard mathematical form of Equation 1 provides reasonable certainty for extrapolating the data of Harkins and Jordan to the considerable degree required for application of the ring method to interfacial tension measurements.

Figure 1 presents the correction factor, F, as a function of the quantity $P/(D-d)$. Two sets of curves are given, the upper set applicable to a ring of circumference equal to 4 cm. and the lower set to one of 6 cm. Individual curves in each set apply to cases of definite values of R/r in the following manner:

For the lowest curves in each set, $R/r = 30$
For the second lowest curves in each set, $R/r = 40$
For the third curves from bottom in each set, $R/r = 50$
For the top curves in each set, $R/r = 60$

The curves were calculated from Equations 1 and 2.

It is indicated by the curves of Figure 1 that the correction values for a 6-cm. ring are much smaller than those for the 4-cm. ring. Upon this basis it follows that, in the interests of greater precision, the larger ring should be used, since the required degree of extrapolation for the correction factor is considerably less than for the smaller ring and the actual magnitudes of F are also smaller.



The validity of Equations 1 and 2 has been checked by means of measurements with two du Nöuy tensiometers (using 4- and 6-cm. rings) as well as by an independent method—namely, the capillary method as described by Bartell and Miller (*1*). Nine oils have been studied. A brief description of these oils is given in Table II, and interfacial tension data are shown in Table III.

These data show good agreement between the results of the capillary method and corrected results obtained by the ring method for 8 of the 9 oils shown above; and in the case of sample E, which has such a high density that $P/(D-d)$ is 922 and 1141, respectively, for the 4- and 6-cm. rings used, so that the correction factors are extremely high, the agreement is fair. The value for benzene is in good agreement with the value of 34.68 at 25° C. reported in the literature (*3*). For samples whose density approaches that of water, $P/(D-d)$ becomes very large and the ring method loses precision. Oils ordinarily encountered usually have a density of less than 0.90 and are nearly always under 0.95, however, and in this range the ring method, modified by the above outlined correction, is capable of reasonably good precision and accuracy.

Table II. Oils Used for Correlation of Interfacial Tension by Ring and Capillary Methods

Oil	Description	Density at 25° C.
A	Specially treated kerosene cut	0.7825
B	Solvent-extracted transformer oil	0.8501
C	50-50 blend of A and B	0.8171
D	Untreated medium distillate cut from recycled stock taken from cracking unit	0.8469
E	Untreated heavy distillate cut from recycled stock taken from cracking unit	0.9765
F	67-33 blend of D and E	0.8907
G	33-67 blend of D and E	0.9334
H	Solvent-extracted 150 viscosity turbine oil	0.8843
I	Benzene	0.8735

Table III. Comparison of Ring and Capillary Methods for Determining Interfacial Tension of Oil against Water

(Temperature, 25° C.)

	S (Uncorrected)				F		S (Corrected)		
	4-Cm. Ring		6-Cm. Ring						
Oil	R/r = 41.7	R/r = 38.8	R/r = 54.0	R/r = 53.4	4-cm. ring	6-cm. ring	4-cm. ring	6-cm. ring	Capillary method
A	42.0	..	45.5	..	1.153	1.041	48.4	47.4	47.4
B	41.0	..	45.4	..	1.233	1.097	50.6	49.8	49.2
C	40.4	..	44.9	..	1.182	1.064	47.8	47.8	47.8
D	30.7	..	34.2	..	1.161	1.050	35.6	35.9	35.8
E	19.0	..	23.5	..	1.642	1.414	31.2	33.2	30.7
F	25.1	..	27.0	..	1.193	1.067	29.9	28.8	29.4
G	22.4	..	25.1	..	1.295	1.141	29.0	28.6	29.2
H	..	42.4	..	48.8	1.311	1.159	55.6	56.6	56.0
I	..	29.1	..	32.6	1.189	1.072	34.6	34.9	34.6

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Determination of Wax in Asphaltic Products

A Propane-Hexone Method

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THE presence of wax in asphaltic products is well known since it can frequently be detected by the hazy appearance of the asphalt surface. Its association with service behavior and source of the asphalt has naturally been assumed from the fact that its presence is readily recognized.

The early methods for the determination of wax in asphaltic products employ high-temperature distillation, vigorous chemical treatment, or selective adsorption to remove or transform the asphaltene-resin fraction, this being necessary before the wax can be determined by crystallization methods (*1*). Of these procedures the Holde method, which includes a destructive distillation of the sample to coke, is the best known and most widely used. The possibility that some of the solid paraffins in asphalt may be decomposed or altered by such vigorous treatment has long raised doubt as to the reliability of the results obtained for wax content, and the need for a dependable method is reflected in the publications of the past few years (*2*, *3*, *5*, *6*).

The present paper describes a method developed in this laboratory for determining wax in asphalt, which has been used very successfully for several years. The asphalt is treated with propane to separate the asphaltene-resin fraction from the oil-wax fraction, and the wax is subsequently isolated from the oil-wax fraction by low-temperature crystallization from solution in methyl isobutyl ketone (commercial "Hexone").

In this method, advantage is taken of the well-known ability of propane to effect a rapid clean separation of the asphaltic and resinous materials which interfere with the crystallization of the wax in the dewaxing step. The mild conditions of the propane treatment offer little possibility of chemically or physically altering the wax; hence, it is determined in the form in which it actually occurs in the asphalt.

The use of methyl isobutyl ketone for the separation of the wax from the oil fraction has been found very satisfactory, since it possesses the requisite properties of a good wax-crystallizing solvent—namely, high oil solvency, low wax solvency, and good filter rates. These properties of Hexone were shown by Gross and Overbaugh (*4*) in their work on dewaxing lubricating oils.

Equipment

The propane-treating equipment is shown in Figure 1, and the complete assembly for this operation is shown in Figure 2. This apparatus has been constructed so that it can be operated rapidly with complete safety. *A*, the precipitation vessel, is surrounded by a jacket through which hot water or steam is circulated to obtain the desired precipitation temperature of 68.3–71.1° C. (155–160° F.). The copper tube which extends nearly to the bottom of *A* has a removable filter at the end which is made up of a 40-mesh copper screen, canvas cloth, filter paper, canvas cloth, and 40-mesh copper screen. Also attached at the bottom end of the filter tube and fitting inside the cylinder are two 40-mesh screen disks 1.27 cm. (0.5 inch) apart. The function of these disks is to collect the bulk of the precipitate and thereby prevent it from settling on the bottom of *A*, where it would tend to plug the filter. *B* is used as a transfer vessel and consists of a special high-pressure Pyrex glass tube fitted in a brass container with observation slots; it operates under a pressure of 123,000 kg. per sq. meter (175 pounds per square inch) and serves to collect the propane-soluble matter (oil and wax).

The equipment for the dewaxing step is substantially the same as that employed by other investigators (*1*). Two methods of chilling have been used—namely, (1) a refrigerated brine-cooled bath which contained sheathed openings 44 by 140 mm., suitable for standard 120-ml. (4-ounce) bottles and a conical opening for the filtering funnel, and (2) a kerosene bath chilled with solid carbon dioxide. In either method the temperature was readily maintained at −17.8° or −26° C. (0° or −15° F.), as desired.

Materials

Methyl isobutyl ketone (Hexone), and commercial propane.

Deasphalting and Deresining

PREPARATION OF SAMPLE. Weigh a 50-cc. beaker and stirring rod approximately, add 5.20 to 5.50 grams of asphalt sample, and record the gross weight of beaker, stirring rod, and sample to the second decimal. Add 11.0 ml. of benzene and weigh to nearest 0.1 gram; cover and warm on a steam bath until solution is complete. Reweigh, and if the heating procedure has reduced the amount of benzene to less than 8.8 grams, add benzene to bring the weight of benzene to this figure, which is equivalent to 10 ml.

Then pour the benzene solution into *A* (Figure 1), avoiding splashing the walls. Place the beaker and stirring rod with adhering solution on the steam bath during the course of the remaining operations and finally weigh (±0.01 gram) after the benzene has been completely evaporated.

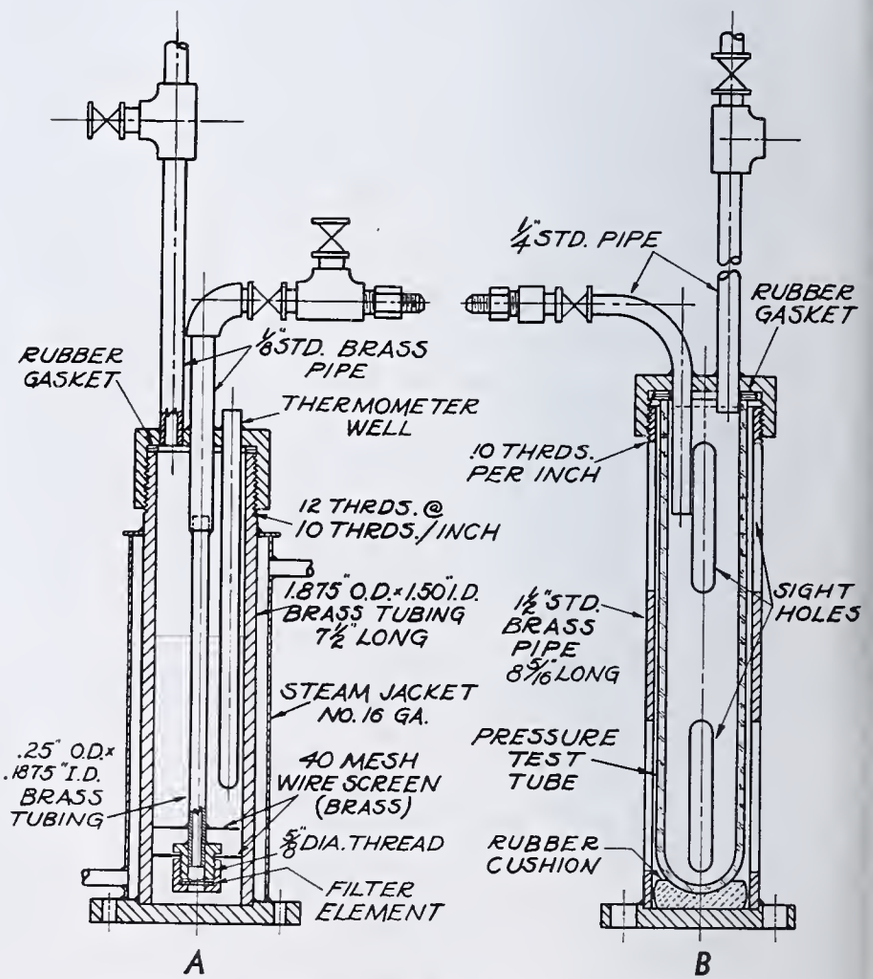

FIGURE 1. APPARATUS FOR TREATING WITH PROPANE
A. Precipitation vessel. *B*. Transfer vessel

Propane Treatment (Figure 2). The benzene solution of the sample having been transferred to *A*, screw the cover tightly into place and introduce 100 ml. of propane from the graduated propane reservoir, *XY*, compressed nitrogen gas serving to assist in the transfer. Nos. 1 through 14 of Figure 2 designate the various valves used in this assembly.

After closing the valves on *A*, detach it and thoroughly mix its contents by inverting and shaking for 30 seconds. Again attach to the propane line, open valve 8, and place a thermometer in the well. Connect a steam line to the jacket inlet (top) and pass steam through until the temperature in the vessel reaches 68.3° to 71.1° C. (155° to 160° F.). The pressure in *A* will be at approximately 246,000 kg. per sq. meter (350 pounds per square inch) at this temperature. With these conditions maintained, allow the precipitated asphaltenes and resins to settle for 5 minutes or longer, after which transfer the propane solution of oil and wax, while still being heated, to *B* in the manner of a pressure decantation. Completion of the transfer will be indicated by the approximate volume in *B*, but primarily by the rapid drop of pressure in *A*. When the pressure in *A* drops to 211,000 kg. per sq. meter (300 pounds per square inch), 35,000 kg. (50 pounds per square inch) pressure of nitrogen is superimposed on the contents of *A* through the connections leading to valve 13. Wash the contents of *A* once with 100 ml. of propane and during this washing operation allow the contents of *B* to evaporate slowly. When the contents of *A* have settled, pressure-decant the propane washings which it contains to *B*, from which the propane is permitted to escape as before. Remove test tube and wash contents into dish, using benzene as solvent, evaporate on steam bath, and record weight. This residue (oil plus wax) is then ready for the wax analysis.

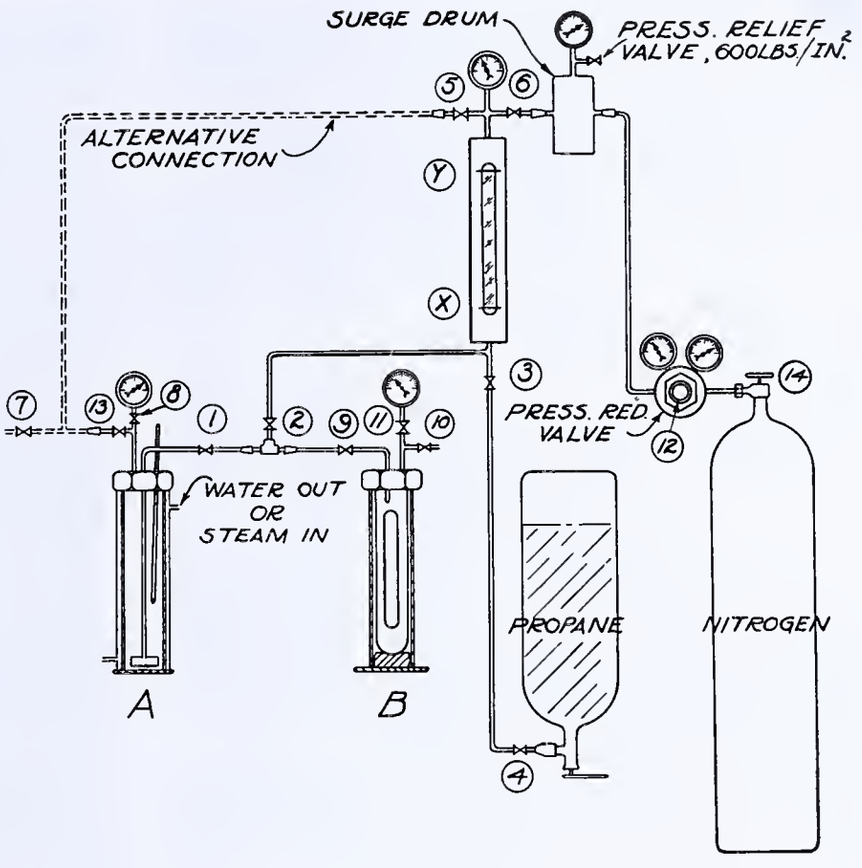

Figure 2. Assembly for Treating with Propane

Dewaxing

Warm 2 to 3 grams of this residue on a steam bath to approximately 65.5° C. (150° F.). At the same time, warm 75 ml. of Hexone to approximately the same temperature, then add enough of it to dissolve the sample. Transfer the dissolved sample to a 120-ml. (4-ounce) bottle and wash the dish into it with the remaining warm Hexone. Cork and shake thoroughly to mix the sample. Warm the solution to 65.5° C. (150° F.) on a steam bath, then allow to cool to room temperature, before placing in an ice bath for 20 to 30 minutes. After precooling in this manner, bring the mixture to a temperature of −17.8° C. (0° F.) by immersing in a suitable bath; an hour in a bath a few degrees below −17° C. (0° F.) has been found satisfactory.

Suction-filter the precipitated wax through Whatman's No. 42 (12.5-cm.) filter paper, wetted with cold Hexone, on a funnel at −17.8° C. (0° F.). Shake the stoppered bottle containing the wax-solvent mixture gently to break the solid structure and carefully pour into the filter, avoiding suction to dryness before washing. When the bulk of the mixture has been transferred to the funnel, discontinue the suction before "cracks" are formed in the wax cake. Wash the sample bottle immediately and thoroughly with approximately 20 ml. of cold Hexone at −17.8° C. (0° F.), and transfer the washings to the filter with the bulk of the wax, saving the bottle with any adhering wax. Again apply suction and continue until the wax is free of solvent. Remove the paper and wax and dissolve this wax, as well as that which was left adhering to the bottle, through the paper with hot benzene. Evaporate the benzene solution on a steam bath, dry the residue at 105° C. (221° F.) for 30 minutes to remove traces of benzene and Hexone, cool in a desiccator, weigh, and calculate the wax back in terms of the original asphalt sample.

Results

The potential utility of methyl isobutyl ketone as a solvent in the quantitative determination of wax was suggested by experiences with its use in dewaxing lubricating oils, which showed that it has excellent oil-solvent properties and low wax-solvent properties, and gives good filter rates (*4*).

Its suitability as a solvent for the analytical determination of wax in asphalt products was investigated by testing synthetic oil-wax samples.

The oil-free wax component of these samples was prepared from a coastal residuum by deasphalting it with propane, dewaxing the deasphalted oil with a solvent consisting of 40 per cent methyl ethyl ketone and 60 per cent benzene at −26.1° C. (−15° F.), and twice deoiling the wax at 10 to 1 dilution in the same mixed solvent. This wax was considered to be substantially oil-free and had a melting point of 69.4° C. (157° F.).

Preliminary tests were carried out to determine the most suitable solvent-oil ratio. For this purpose, 5 per cent of the oil-free wax was added to a high-viscosity propane deasphalted, dewaxed, and solvent-refined residual oil. Dewaxing tests were made in which the size of the sample was varied, but the volume (75 ml.) of methyl isobutyl ketone kept constant.

These results are shown in Table I, the first four tests of which were made on the blank oil and show that oil separation will occur if the solvent-oil ratio is too low. Similar tests made on the 5 per cent wax-oil sample (correction being made for the blank oil) indicate that the best accuracy is obtained with 2- to 3-gram samples. With dewaxing sample weights in this range, the melting point of the recovered wax was 65.6° C. (150° F.), as compared with 69.4° C. (157° F.) on the original wax, illustrating the good separation of wax and oil obtained with this solvent. The results show that with samples of

Table I. Effect of Sample Size on Accuracy of Wax Separation Step

	Methyl Isobutyl Ketone (75 Ml.)					
Charge Material	Weight of sample	Wax found[a]	Wax found less blank	Error	Melting point of "found" wax	
	Grams	%	%	%	° C.	° F.
Refined residual oil (no added wax)	0.3	0.4	..	..	....	
	1.0	0.5	..	..	....	
	3.0	0.8	..	..	....	
	5.0	2.3	..	..	Oil separation	
Refined residual oil (plus 5% of 157° F. melting point wax)	0.3	2.5	2.1	−2.9	....	
	1.0	4.9	4.4	−0.6	....	
	2.0	5.7	5.0	0.0	65.6	150
	3.0	5.6	4.8	−0.2	....	
	5.0	9.4	7.1	2.1	54.4 (*circa*)	130

[a] Wax determination made at −26.1° C. (−15° F.).

Table II. Determination of Wax in Synthetic Wax-Asphalt Samples

(Propane-Hexone Method)

Nature of Sample	Wax Added (157° F. Melting Point)	Wax Content Found[a]	Wax Content Calculated (Blank plus Wax Added)	Error
	%	%	%	%
Dewaxed residuum (0.8% wax)	1.1	2.0	1.9	0.1
	2.9	3.2	3.7	−0.5
	5.0	5.4	5.8	−0.4
	5.0	5.8	5.8	−0.0
	5.0	5.7	5.8	−0.1
Air-blown asphalt 1 (1.4% wax)	2.1	3.4	3.5	−0.1
Air-blown asphalt 2 (4.7% wax)	2.4	7.0	7.1	−0.1
Air-blown asphalt 3 (5.8% wax)	2.3	7.7	8.1	−0.4

[a] 2.5 grams of oil-wax material dissolved in 75 cc. of methyl isobutyl ketone and chilled to −26° C. (−15° F.).

2 to 3 grams the yield of wax was almost theoretical. On the basis of these results, 2- to 3-gram oil-wax samples and 75 ml. of solvent were used in subsequent work. Smaller samples may give low results, while the use of large samples gives high results that are due to oil separation which is reflected by the low melting point of the separated wax.

The method was then applied to a series of synthetic wax-asphalt samples made by adding known amounts of the oil-free wax to a number of asphaltic products, including residua and air-blown asphalts of high melting point. In each case, the synthetic sample was fractionated with propane in the manner described and the wax determined on the oil-wax fraction. The asphaltene-resin fraction which is removed in the first step by the propane is a black brittle solid, while the oil-wax fraction is usually a dark green oil. The data on these synthetic samples are given in Table II. The "wax content calculated" is the per cent wax in the asphalt plus the wax added, and is compared with the "wax content found" to obtain "error". The results illustrate the accuracy and reproducibility of this method on asphalts of low and high wax content. It gives results which are slightly lower than the theoretical wax content based on the blank test and the amount of added wax. In the case of dewaxed residuum, the melting point of the recovered wax ranged from 63.9° to 68.3° C. (147° to 155° F.), as compared to 69.4° C. (157° F.) for the original added wax. The difference is probably due to the separation of low-melting wax by the methyl isobutyl ketone which was not separated in the methyl ethyl ketone–benzene dewaxing of the residuum or to the occlusion of small amounts of oil.

To reduce the time of analyses by this method, several experiments were made to determine the effect of settling time in the deasphalting step. The time was varied from 5 to 30 minutes; the results (Table III) show that a short period of settling is entirely satisfactory and 5 minutes has been adopted for routine use. This series of wax determinations was made at −17.8° C. (0° F.). This temperature gave entirely satisfactory results and was adopted for general routine use, since it was more easily attained than −26.1° C. (−15° F.).

In view of the wide use of the Holde method for the determination of wax in asphaltic products, results (Table IV) by it have been compared with those by the present method on samples of asphalt to which were added amounts of wax. In these tests the average error of the propane–methyl isobutyl ketone method was −0.1 per cent, as compared with an average error of −4.1 per cent by the Holde method. Despite the higher wax content by the present method, its melting point is also higher, which is proof that the increased wax content is not due to oil separation but rather to the separation of existing solid paraffins which are pyrolytically decomposed and lost in the distillation step of the Holde method. These results indicate the present method to be more suitable for the determination of the naturally occurring wax in asphalt.

Consideration was given to substituting methyl isobutyl ketone for alcohol-ether in the Holde method. However, preliminary tests gave results for wax content which were even lower than when alcohol-ether was used. These results indicated that the so-called wax by the Holde method may contain oil.

The use of alcohol-ether for determination of wax in the propane-treated oil-wax fraction was also considered. In preliminary tests, excessive oil separation was obtained and further experiments were discontinued. The results illustrated well the superiority of methyl isobutyl ketone over alcohol-ether as solvent in the determination of wax.

Table III. Effect of Deasphalting Settling Time on Wax Determinations

(Propane-Hexone Method)

Settling Time	Wax Found[a]	Melting Point of Wax	
Min.	%	° *C.*	° *F.*
	Sample 1		
5	2.33	61.1	142
10	2.02	61.7	143
20	2.22	61.1	142
30	2.42	58.3	137
	Sample 2		
5	6.67	61.7	143
10	5.98	61.1	142
20	6.50	61.7	143
30	6.61	59.4	139

[a] Dewaxing test made at −17.8° C. (0° F.).

Table IV. Comparison of Wax[a] Content by Propane-Hexone and Holde Methods

Nature of Sample	Wax Added (157° F. Melting Point)	Wax Content Calculated (Blank plus Wax Added)	Propane-Hexone: Wax content found	Propane-Hexone: Error	Propane-Hexone: Melting point ° C.	Propane-Hexone: Melting point ° F.	Holde[b]: Wax content found	Holde[b]: Error	Holde[b]: Melting point ° C.	Holde[b]: Melting point ° F.
	%	%	%	%			%	%		
Dewaxed residue	None	0.8	0.8	..	..	..	0.6	..	..	..
	5.0	5.8	5.6	−0.2	61.7	143	2.2	−3.6	58.3	137
Air-blown asphalt 1	None	1.4	1.4	..	58.9	138	1.6	..	..	..
	2.1	3.5	3.4	−0.1	65.6	150	..	..	..	..
	5.0	6.4	..	..	..	..	2.5	−3.9	57.8	136
Air-blown asphalt 2	None	4.7	4.7	..	66.1	151	1.0	..	..	..
	2.4	7.1	7.0	−0.1	..	..	..	..	..	..
	5.0	9.7	..	..	..	..	4.9	−4.8	57.8	136

[a] Wax determinations made at −26.1° C. (−15° F.).
[b] Blank corrections applied to results by Holde method are based on blanks obtained by proposed method, because the latter gave higher yields of wax of higher melting points, pointing to probable true wax content.

Method of Test for Melting Point of Wax

The following procedure has been found satisfactory for determining the melting point of the small samples of wax that are obtained in its determination. It is a refinement of a method long used in wax plants.

Apparatus. Pour test jar (A. S. T. M.), test tube, 15 × 1.88 cm. (6 × 0.75 inches), thermometer, and 600-ml. beaker.

Procedure. Melt the wax on a steam bath and wet the cold thermometer bulb with the molten wax to a depth of about 3 mm. Remove promptly, so that the layer of wax which adheres to the

bulb weighs between 8 and 12 mg. A little experience soon teaches when the correct weight has been applied. If too much sample is taken, it will drop from the bulb when subsequently melted; if too little, the subsequent congealing will be difficult to observe.

With a cork fix the thermometer in the test tube, so that the bottom of the bulb is 12 mm. above the bottom of the tube. With a cork fix the test tube in the pour test bottle which is full of cold water (15° C.); the cork should have a small slit in it to permit gas to escape. Place the pour test bottle in a water bath kept at 75° C., or at least 5° C. above the melting point of the wax.

After the wax has melted to a clear liquid, carefully remove the assembly from the bath and tilt from the vertical to a horizontal position once every 0.3° C. drop in temperature, observing the drop of wax on the bulb of the thermometer.

The temperature at which this drop of wax solidifies, as indicated by the absence of movement on tilting, is the end of the determination. The last temperature at which movement was observed on tilting the assembly is recorded as the melting point of the sample. Experience has shown that results can be reproduced to within 0.3° C.

Summary

This method comprises separating the asphaltene-resin fraction from the oil-wax fraction by hot liquid propane under pressure and determining the wax in the oil-wax fraction by crystallization at low temperatures from solution in methyl isobutyl ketone (Hexone).

The propane treatment effects a clean removal of the asphaltene-resin fraction, the presence of which interferes generally with crystallization methods for wax, and avoids the detrimental effects of more drastic pretreatments, such as digestion with strong sulfuric acid or destructive distillation, which may destroy some of the wax.

Hexone possesses the necessary properties of an outstanding reagent for wax determination—namely, high oil-solvent power, low wax-solvent power, and good filtration rates.

Application of the method to asphaltic samples containing known additions of wax indicate that the method is accurate. Wax contents are generally higher than those found by the Holde method.

Acknowledgment

The authors wish to acknowledge their appreciation to David S. Stairs, who made many of the separations and determinations, and to P. L. LaTour, who assisted in the formal arrangement of the method.

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Indiana Stirring Oxidation Test for Lubricating Oils

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ATTEMPTS to develop laboratory tests suitable for predicting the oxidation stability of lubricating oils have been numerous since the first widely used test was published by Sligh (*7*) in 1924. Since that time some eighty papers have appeared either describing new laboratory tests or criticizing the old ones. The Indiana oxidation test (*1*, *5*), published in 1934, was extensively used for evaluating the stability of conventional oils. However, Maverick (*3*) and Rogers (*6*) subsequently pointed out that the test is not suitable for the more recently developed compounded oils, such as those containing inhibitors and detergents. Another well-known test, first appearing several years ago, is the Underwood oxidation test (*8*), which was first developed to evaluate bearing corrosion and was later modified to be applicable to the study of oxidation stability (*4*).

The general trend of opinion in the last several years has been to minimize the value of laboratory oxidation tests and to place more and more emphasis on the full-scale engine test. Notwithstanding the essential correctness of such a point of view, it remains so extremely important to have some simple and rapid method of predicting oil stability, especially in the development of improved oils, that the laboratory test has continued to receive a generous share of attention. This paper discusses a new laboratory testing technique which has been found valuable for predicting the oxidation stability of both inhibited and uninhibited crankcase lubricating oils.

A specific example of a compounded oil that was not correctly evaluated by the Indiana oxidation test was one of the first proposed heavy-duty lubricating oils—namely, one containing calcium naphthenate as detergent and a sulfurized saponifiable compound as inhibitor. Numerous engine tests had shown that the addition of either the inhibitor alone or the inhibitor plus the detergent was highly effective in improving the cleanliness of engines operated under severe test conditions. The Indiana oxidation test indicated that the compounded oils were far less stable than the control oil. Furthermore, compared to the extent of deterioration occurring in accelerated engine tests, the Indiana oxidation test could scarcely be called an accelerated test. These results are clearly shown in Table I.

Table I. Effect of Sulfurized Saponifiable Additive and Calcium Naphthenate

[Indiana oxidation tests. Summarized test conditions: 10 liters of air per hour blown through 300 cc. of oil at 171.7° C. (341° F.)]

	Sludge			Acidity		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
	Mg./10 g. oil			*Mg. KOH/g. oil*		
Oil A	0	0	0	0.8	1.6	...
Oil A + 3.0% sulfurized saponifiable additive	0	11	376	1.5	3.7	...
Oil A + 3.0% sulfurized saponifiable additive + 0.25% calcium naphthenate	19	161	400	1.7	2.8	3.3
Oil B	0	45	...	...	...	...
Oil B + 3.0% sulfurized saponifiable additive + 0.25% calcium naphthenate	10	300	...	1.7	3.7	...

Table II. Effect of Sulfurized Saponifiable Additive and Calcium Naphthenate

[Underwood oxidation tests[a]. Summarized test conditions: 1500 cc. of oil at 163° C. (325° F.) are recirculated through an apparatus primarily of iron construction. The oil is sprayed through several nozzles at a pressure of 700 grams (10 pounds) per sq. inch against a 5 × 25 cm. (2 × 10 inch) copper baffle, one stream of oil impinging on a hard metal bearing.]

	Sludge		Acidity	
	5 hours	10 hours	5 hours	10 hours
	Mg./10 g. oil		*Mg. KOH/g. oil*	
No Added Catalyst				
Oil B	73	600	5.2	10.1
Oil B + 3.0% sulfurized saponifiable additive + 0.25% calcium naphthenate	18	25	0.4	0.4
0.016% Fe_2O_3 as Iron Naphthenate				
Oil A	78	564	8.9	12.8
Oil A + 3.0% sulfurized saponifiable additive	41	60	0.94	1.0
0.010% Fe_2O_3 as Iron Naphthenate				
Oil B	204	1380	10.1	16.8
Oil B + 3.0% sulfurized saponifiable additive + 0.25% calcium naphthenate	56	81	0.8	0 9

[a] By courtesy of General Motors Research Laboratory.

On the other hand, the Underwood stability test agreed with the engine in showing that the compounded oils were more desirable and showed a remarkably accelerated rate of deterioration as compared to the Indiana oxidation test. Results of these tests are shown in Table II.

The results of the Indiana oxidation test are so far out of line with actual engine and Underwood test results that it seems apparent that it must neglect some important factor contributing to oil deterioration, present in actual engine operation and, at least to some extent, in the Underwood test. The latter test is a workable and useful laboratory tool for predicting engine performance. However, it was desired to develop a simpler test in which all the conditions contributing to oxidation could be more closely controlled.

It was thought preferable to examine one by one some of the more outstanding conditions involved in engine tests that would be expected to contribute to oil deterioration. If one or more test conditions could be isolated as being responsible for the rapid oil deterioration and for correlation with the engine in the case of the oils previously mentioned, it would be possible to devise a simple test incorporating these particular conditions.

Development of Stirring Test Technique

Two specific objectives were sought in this study: (1) to line up the control oil and the oil containing sulfurized additive so as to show the latter more stable towards deterioration, and (2) to obtain a much more accelerated rate of deterioration of the control oil than is observed in the Indiana oxidation test. The most striking of the conditions present in engine tests are shown below, with a description of the results obtained when this test condition was emphasized.

A large amount of metallic catalyst is present in engine tests. An enormous amount of metallic iron is exposed to the oil in addition to the smaller amounts of copper present in bearings, bushings, and tubing. However, simply adding iron and copper catalysts to the Indiana oxidation test caused little improvement in test results. Data from these tests are shown in Table III for the control and inhibited oils.

In engine tests, a small part of the oil is continuously exposed in thin films on the various parts of the piston and on other parts of the engine above the oil level in the crankcase. To examine this effect, an apparatus was constructed whereby oil at a bulk temperature of 171.7° C. (341° F.) was recirculated by spraying over a copper plate at 179.4° C. (355° F.). The rate of oil circulation was such that only a thin film of oil covered the copper plate at all times. This procedure did not cause appreciably accelerated deterioration of the oil, and did not show the compounded oil as superior to the control oil.

Table III. Effect of Sulfurized Saponifiable Additive

[Indiana oxidation tests in presence of metallic catalysts. Summarized test conditions: 10 liters of air per hour blown through 300 cc. of oil at 171.7° C. (341° F.)]

	Sludge		Acidity	
	24 hours	48 hours	24 hours	48 hours
	Mg./10 g. oil		*Mg. KOH/g. oil*	
0.112% Iron Naphthenate (0.016% Fe_2O_3) as Catalyst[a,b]				
Oil A	0	0	3.3	6.1
Oil A + 3.0% sulfurized saponifiable additive	0	10	3.6	6.5
0.112% Copper Naphthenate as Catalyst[c,b]				
Oil A	0	0	3.6	2.1
Oil A + 3.0% sulfurized saponifiable additive	124	181	5.9	3.9
10 Sq. Inches of Metallic Copper per 300 Grams of Oil as Catalyst[d,b]				
Oil A	0	13	3.7	6.2
Oil A + 3.0% sulfurized saponifiable additive	0	190	3.5	4.3

[a] Tests repeated with 10 times the amount of iron naphthenate.
[b] Deterioration only slightly accelerated. Same relative stabilities of the oils.
[c] Tests repeated with 10 times the amount of copper naphthenate.
[d] Tests repeated with 10 times the amount of copper.

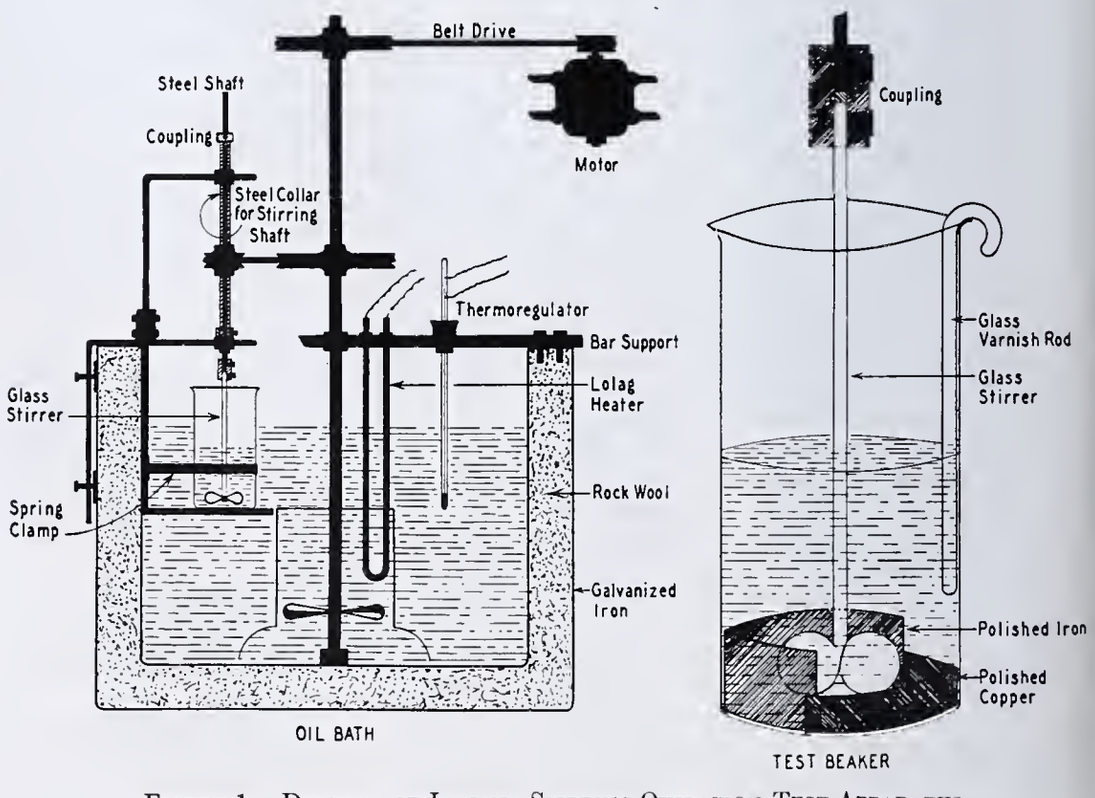

Figure 1. Diagram of Indiana Stirring Oxidation Test Apparatus

TABLE IV. EFFECT OF SULFURIZED SAPONIFIABLE ADDITIVE AND CALCIUM NAPHTHENATE

[Indiana stirring oxidation tests. Summarized test conditions: 500-cc. beaker, 250 cc. of oil, 5 sq. inches of Cu, 10 sq. inches of Fe, 1300 r. p. m., 165.5° C. (330° F.)]

	Increase in Saybolt Viscosity at 100° F. in 48 Hours	Sludge		Acidity		Varnish Equivalent to Standard Rods	
		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
	Sec.	*Mg./10 g.*		*Mg. KOH/g.*			
Oil A	1210	356	958	6.8	10.2	5	2
Oil A + 3.0% sulfurized saponifiable additive	253	29	150	2.2	2.8	9–10	8
Oil A + 3.0% sulfurized saponifiable additive + 0.25% calcium naphthenate	354	20	160	2.8	7.0	10	10

TABLE V. EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHORUS INHIBITOR

[Indiana oxidation tests. Summarized test conditions: 10 liters of air per hour blown through 300 cc. of oil at 171.7° C. (341° F.)]

	Sludge			
	24 hours	48 hours	72 hours	96 hours
	Mg./10 g. oil			
Oil A	0	0	3	42
Oil A + 0.25% phosphorus inhibitor	14	20	29	55
Oil A + 0.50% phosphorus inhibitor	23	40	51	74
Oil A + 1.0% phosphorus inhibitor	27	41	53	72

In engine tests, oil is subjected to very high temperatures on the piston walls and in the ring grooves. It is generally thought that a large part of oil deterioration in the engine takes place in the region of these local high temperatures, rather than in the crankcase. The Indiana test was modified so that a small part of the oil was continuously subjected to a metal surface at a considerably higher temperature than the bulk of the oil. However, no favorable change in test results was noted.

Aeration is obtained in the engine not by blowing a stream of air through the oil, as in the Indiana oxidation test, but by beating the air into the oil through the action of the rapidly turning crankshaft and by further contact of air with the oil in the splash or forced-feed lubrication system. The conditions are such in the engine that the volatile oxidation products are to a large extent retained in the oil. The importance of this factor was first emphasized in relation to bearing corrosion by Davis (*2*). As a first means of evaluating the importance of this factor, Indiana oxidation tests were run on the control oil and on the compounded oils, in which the volatile products were returned to the bulk of the oil by means of a condenser. Test results were unsatisfactory and similar to those obtained from the regular Indiana oxidation test. However, an interesting observation was made when this type of test was run with copper wire in the condenser. The volatile products from the control oil rapidly attacked the copper, even at the relatively low temperatures prevalent in the condenser, causing the formation of green copper soaps; whereas the oil containing the sulfurized inhibitor gave off an appreciable amount of hydrogen sulfide, coating the copper and preventing the attack of the copper by the volatile oxidation products. This would explain the failure of the addition of metallic catalysts to the regular Indiana oxidation test to accelerate deterioration significantly and to show the inhibited oil in a favorable light. Blowing with air removes the volatile oxidation products before they have a chance to exert their effect on the metallic catalysts and thus the full harmful effect of the metal catalysts is never experienced.

As an alternative method of keeping the volatile oxidation products in the oil, tests were devised wherein the oil was aerated by rapid stirring, rather than by blowing with air.

In the test conditions hereinafter referred to as the Indiana stirring oxidation test, the following test conditions have been used:

Sample, 250 cc. of oil in a 500-cc. tall-form beaker
Temperature, 165.5° C. (330° F.)
Catalyst, 32.26 sq. cm. (5 sq. inches) of copper surface, 64.52 sq. cm. (10 sq. inches) of iron surface
Aeration, stirring at 1300 r. p. m. with a glass stirrer (two blades, rounded, 2 × 2 cm., at a 40° pitch)

Figure 1 is a diagrammatic sketch of the apparatus used. Actually the oil bath is arranged so that fan tests can be run simultaneously. It is necessary to keep the oil bath at 341° F. to maintain the oil in the test beaker at 330° F.

This test shows that the compounded oil is appreciably better than the control oil and that greatly accelerated deterioration of the control oil has occurred. Results of the Indiana stirring oxidation test on these oils are shown in Table IV.

In the Indiana stirring oxidation test, four deterioration products of the oil are measured. The viscosity, sludge, and acidity values are self-explanatory, as shown in Table IV. The varnish values are obtained by suspending glass rods in the oils under test. One rod is removed every 24 hours, rinsed with naphtha, carefully wiped free of any loosely adhering sludge with cotton, and visually compared to a set of ten standard varnish rods. A rating of 10 signifies a perfectly clean, varnish-free rod; a rating of 1 signifies a rod heavily coated with varnish. Intermediate ratings signify varnish to an extent represented by the proximity of the rating to 10 or 1.

In Table IV it may be noted that the addition of the inhibitor alone causes greatly improved over-all stability. A further addition of the detergent causes still greater improvement in varnish, but has a slight detrimental effect on acidity and viscosity development. However, some detergents, in addition to improving varnish characteristics, actually cause an improvement in the over-all stability of the oil.

It was realized that a laboratory test correlation with engine performance, based on one short series of oils, was of little importance if not supported by further data. Numerous accelerated engine stability tests had shown that a particular phosphorus-type inhibitor caused improved performance as regards engine cleanliness and oil stability in proportion to the concentration of the inhibitor used. This improved performance was certainly not predicted by Indiana oxidation tests as shown in Table V. However, when this series of oils was run in the Indiana stirring oxidation test, the oils showed improved stability in all respects in proportion to the amount of inhibitor used (Table VI).

TABLE VI. EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHORUS INHIBITOR

[Indiana stirring oxidation tests. Summarized test conditions: 500-cc. beaker, 250 cc. of oil, 5 sq. inches of Cu, 10 sq. inches of Fe, 1300 r. p. m., 165.5° C. (330° F.)]

	Increase in Saybolt Viscosity at 100° F. in 48 Hours	Sludge			Acidity			Varnish Equivalent to Standard Rods		
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
	Sec.	*Mg./10 g.*			*Mg. KOH/g.*					
Oil A	1210	356	958	...	6.8	10.2	...	5	2	...
Oil A + 0.25% phosphorus inhibitor	833	56	420	730	4.5	5.6	...	6	2–3	...
Oil A + 0.50% phosphorus inhibitor	758	26	320	600	2.3	4.0	...	8	3	...
Oil A + 1.0% phosphorus inhibitor	116	8	12	20	0.8	1.5	2.1	10	9	9

TABLE VII. COMPARISON OF TESTS ON THE CONTROL AND INHIBITED OILS WITH AND WITHOUT METAL CATALYSTS

[Indiana stirring oxidation tests. Summarized test conditions: 500-cc. beaker, 250 cc. of oil (5 sq. inches of Cu, 10 sq. inches of Fe)[a], 1300 r. p. m., 165.5° (330° F.)]

	Catalyst	Saybolt Viscosity, 100° F., Increase		Sludge				Acidity				Varnish Equivalent to Standard Rods			
		48 hours	96 hours	24 hours	48 hours	72 hours	96 hours	24 hours	48 hours	72 hours	96 hours	24 hours	48 hours	72 hours	96 hour
		Sec.	*Sec.*	*Mg./10 g.*				*Mg .KOH/g.*							
Oil A	No metals	160	221	0	0	0	0	0.8	1.0	1.1	1.1	10	10	10	9
Oil A	With metals	1210	...	356	958	...	...	6.8	10.2	...	...	5	2	...	...
Oil A + 3.0% sulfurized saponifiable additive	No metals	220	...	...	285	399	...	...	1.7	5.6	...	...	9	7–8	...
Oil A + 3.0% sulfurized saponifiable additive	With metals	253	...	29	150	310	...	2.2	2.8	7.5	...	9–10	8	6	...

[a] Used only in tests specified as containing metals.

Theoretical

Because of the need for the use of this test as a practical tool, it has been impossible to investigate the test variables as completely as would be desirable from a scientific viewpoint. However, sufficient background has been obtained to make possible some generalizations regarding what factors are important in the test.

It is not immediately apparent why the stirring test in the presence of metals causes more rapid deterioration than a blowing test at approximately the same temperature, and why it reverses the rate of deterioration observed in blowing tests on uninhibited and sulfur-inhibited oils.

The first natural assumption would be that the rate of deterioration is tied in very closely with the rate of oxygen absorption. This is true only in part in the tests under consideration. Addition of either copper or iron catalysts does cause an increased rate of oxygen absorption, using either the blowing or stirring methods of aeration. The increase is much less where the sulfur inhibitor is present. However, the more rapid rate of deterioration in stirring tests in the presence of metals, compared to that observed in blowing tests in the presence of metals, is not explained by increased oxygen absorption. Oxygen absorption tests were run on the uninhibited oil at 171.7° C. (341° F.) in the presence of the same ratios of metals as used in the Indiana stirring oxidation test with (*a*) aeration by blowing as in the Indiana oxidation test, and (*b*) aeration by rapid stirring as in the Indiana stirring oxidation test. These tests showed essentially the same rate of oxygen absorption in both cases and, in fact, even a slightly lower rate when the stirring technique was used. It seems indicated from somewhat limited data that the more rapid deterioration in the stirring test must be explained by secondary reactions rather than by the primary reaction involving the rate of oxygen absorption.

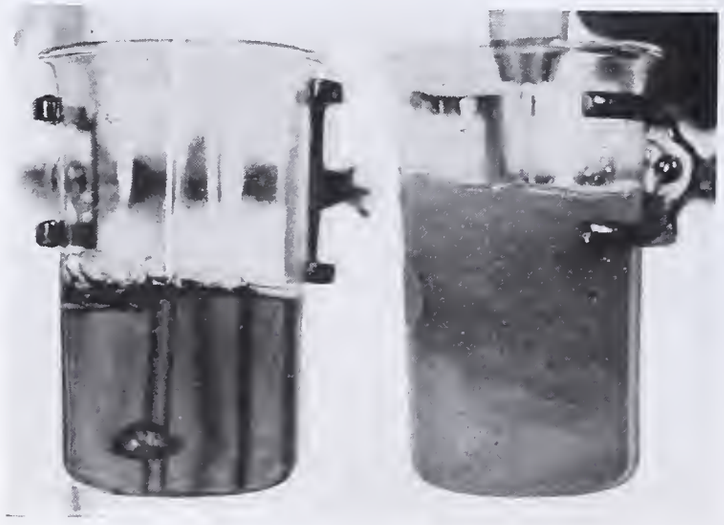

FIGURE 2. BUBBLES IN AIR-BLOWING AND STIRRING TESTS

A logical possibility is that the stirring causes the volatile oxidation products to be retained in the oil for a relatively longer period of time than in blowing tests and that this allows secondary reactions to occur. An examination of the actual mechanics of aeration by stirring compared to aeration by blowing is of interest in this respect. Figure 2 gives an indication of the size of the bubbles involved in an air-blowing test compared with the bubbles in the stirring test.

Since the volatile oxidation products are removed by the bubbles rising through the oil and escaping to the air, the extent of the contact of these gaseous products with the oil and metal catalysts is determined by the surface of the bubbles and by their rate of escape. A rough calculation shows that in stirring tests the gaseous products are in contact with the oil at least several thousand times as long as in blowing tests.

Assuming that this effective retention of volatile products is significant, it still remains to be explained in what way volatile products accelerate deterioration. The first possibility is that, neglecting the presence of the metals, the volatile products react with themselves or with the nonvolatile fraction of the oxidized oil to form the measurable deterioration products. The other possibility is that the volatile products in contact with the metal catalysts undergo secondary reactions, possibly through actual chemical combination with the metal, to give deterioration products. The data in Table VII show that the presence of the metal is necessary for accelerated deterioration of the uninhibited oil to occur and for rating uninhibited *vs.* inhibited oils in accordance with engine performance.

The above facts lead to the generalization that accelerated deterioration of an uninhibited oil in the Indiana stirring oxidation test is due mainly to the effective retention of volatile oxidation products which undergo secondary reactions in the presence of metallic catalysts to cause the formation of the conventionally measured deterioration products, such as sludge, varnish, etc. The relatively good performance of the sulfur-inhibited oil in the same test is apparently due to the deactivation of the metal catalyst by the inhibitor, or to the effects of the inhibitor upon the secondary reactions.

The same generalizations, concerning the relative performance of an uninhibited and a sulfur-inhibited oil in engine tests, are probably applicable. In the engine, the volatile oxidation products are to a large extent retained in the oil and freely contact the extensive metal surface exposed.

Effect of Variables in Test Conditions

Some of the variables in the test conditions have been examined briefly from the standpoint of obtaining orientation as to their effect on rate of deterioration. Stirring speed, amount and type of metallic catalyst, stirring blade pitch and size, and temperature would all be expected to affect the

TABLE VIII. EFFECT OF CHANGE IN TEST CONDITIONS ON DETERIORATION OF CONTROL OIL

Indiana stirring oxidation tests. Test conditions: (unless otherwise specified) 500-cc. beaker, 250 cc. of oil A, 5 sq. inches of Cu., 10 sq. inches of Fe, 1300 r. p. m., 165.5° C. (330° F.)]

Change in Test Conditions	Increase in Saybolt Viscosity at 100° F., in 48 Hours	Sludge			Acidity			Varnish Equivalent to Standard Rods		
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
	Sec.	*Mg./10 g.*			*Mg. KOH/g.*					
No change	1210	356	958	...	6.8	10.2	...	5	2	...
Both metal catalysts omitted	160	0	0	0	0.8	1.0	1.1	10	10	10
Copper omitted	135	0	34	40	0.8	1.7	2.8	10	7	4–5
Iron omitted	1212	705	951	...	6.7	9.5	...	1	1	...
Propeller pitch lowered from 40° to 20°	670	147	820	1140	2.8	6.7	10.4	5	2	...
Stirring rate lowered to 760 r. p. m.	241	118	176	...	1.1	2.8	3.8	9–10	6–7	3
Stirring rate raised to 1740 r. p. m.	2630	327	963	...	8.8	19.0	...	1	1	...

rate of deterioration. Table VIII shows the effect of changes in test conditions. As indicated previously, in the absence of any metallic catalyst, the test is not an accelerated test. In the presence of metallic iron alone as catalyst, the test is somewhat accelerated over that in which no metallic catalyst at all is used, but does not compare with the rate of deterioration noted when copper is present. Stirring with either a low-pitch propeller or at decreased r. p. m. markedly lowers rate of deterioration, and, conversely, increasing the r. p. m. of the stirring rate markedly accelerates deterioration. Since the test results do depend closely on all the test variables, extreme care must be taken to ensure reproducibility of results. However, even in crude form, the test is valuable for showing the marked improvement caused by adding suitable additives to oils.

Application of Stirring Technique to Study of Bearing Corrosion

The stirring test technique has also been found suitable for the investigation of bearing corrosion. Owing in all probability to the more effective retention of the corrosive volatile oxidation products, stirring corrosion tests show much more rapid corrosion than corrosion tests involving aeration by blowing. For example, a section of a cadmium-silver bearing suspended in oil A, under the Indiana oxidation test conditions, shows corrosion only after 80 to 90 hours. Under Indiana stirring oxidation test conditions, appreciable corrosion occurs in less than 20 hours.

The corrosion of copper-lead bearings, even under Indiana stirring oxidation test conditions, did not proceed rapidly. In most cases, after a small amount of initial corrosion, the corrosion stopped entirely. It was assumed that in the case of the more stable oils only the lead was attacked, and that continued corrosion did not occur because only a very small amount of lead could easily contact the oil. In the low stability oils, sufficient corrosive products are formed to corrode the copper, in which case corrosion of the copper-lead bearings proceeds as is expected.

Therefore, in most cases, the corrosion loss of a lead strip has been measured rather than that of a copper-lead bearing.

TABLE IX. APPLICATION OF INDIANA STIRRING OXIDATION TEST TO STUDY OF BEARING CORROSION

Test conditions: lead strip (7 sq. cm. surface) and section of Cu-Pb bearing (5 sq. cm. surface) suspended in oil oxidizing under Indiana stirring oxidation test conditions (additional Cu and Fe catalysts omitted)]

Oil	Lead Strip Corrosion Loss				
	0–1 hour	1–2 hours	2–3 hours	3–4 hours	4–20 hours
	Mg./10 sq. cm. surface				
A	2.3	3.4	9.6	25	530
C	0.4	1.7	11.0	38	554
D	0.6	2.4	12.1	43	1340
E	0.7	0.6	1.0	1.0	67
F	0.4	0.3	0.3	0.4	3.1
G	13.7	7.6	7.7	6.0	15.2
H	30.0	7.3	2.1	1.4	21
I	12.6	9.0	5.7	3.1	42
J	0.9	1.0	1.0	3.1	13
K	11.4	50	46	44	935

Typical lead strip corrosion data are shown for a few oils in Table IX.

Samples A, C, and D represent oils which are not particularly stable toward corrosion caused by oxidation of the oil, although they are probably sufficiently stable for ordinary automotive use. Oils E, F, and J are highly stable toward corrosion and are probably suitable in this respect for heavy-duty service. Oils G, H, and I represent products which are inhibited toward corrosion due to oxidation, but which cause corrosion in the early stages of the test. This is thought to be caused by the acidic nature of the inhibitor itself. It is possible that these oils in long contact with hard metal bearings would cause a harmful amount of corrosion. Oil K contains an additive which is not only corrosive itself but accelerates oxidation of the oil and the corrosion caused thereby.

Thus the stirring corrosion test is not only useful in showing corrosion tendencies due to oxidation of the oil, but also measures the slower corrosion caused by the corrosive nature of the additives themselves.

Value of Stirring Test Technique

This paper is not meant to bring forward a standardized or uniformly applicable laboratory test technique for the evaluation of the oxidation stability of lubricating oils. Rather it means to emphasize the importance of a combination of several oxidation conditions which exist in engine operation, and which are believed to contribute largely toward the extent and nature of oil deterioration in the engine. It is believed that any successful laboratory test should provide for effective retention of volatile oxidation products and contact of these products with metallic catalyst, and that the stirring technique provides the most convenient method for obtaining this effect. Admittedly, in the test described above, some of the conditions are arbitrarily chosen. However, retaining the essential features of the test, it should be possible to select other test variables so that the test is applicable for predicting performance under a wide variety of engine conditions, although it is agreed that the only final evaluation of a crankcase oil is its performance in engines in actual service. It is also believed that the essential features of the test not only may be applicable to the evaluation of crankcase oils, but also may be suitable for predicting performance of industrial oils, turbine oils, and other circulating oils.

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Viscosity Determination of Cellulose

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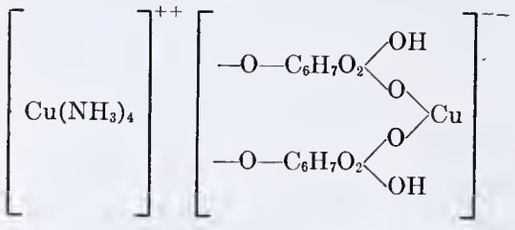
The constitution of cuprammonium cellulose and the ammonia partial pressure of different copper-ammonia compounds with and without the addition of salts have been investigated, as has the influence of oxygen on cuprammonium cellulose solutions. These solutions absorb oxygen, especially quickly in the presence of mercury, and the consequent strong reduction of the viscosity of the cuprammonium cellulose solution makes this method uncertain.

In the viscosity determination of cellulose by its conversion into nitrates the nitration is carried out with phosphoric or glacial acetic acid containing mixed acids. A quick process for nitration and stabilization is described, which produces very little degradation of cellulose and gives easily reproducible results.

THE relationship between the micelle (molecular) weight of cellulose and the viscosity of derivatives has been studied for more than 30 years (*2*, *4*, *8*, *13*, *15*, *17–22*) with more or less success. To determine the viscosity of cellulose the Ost method (*19*), based on its transformation into cuprammonium cellulose, with different modifications, such as that of the Hercules Powder Company (*14*), is used. The author in 1909 (*2*) and in many publications thereafter used conversion into cellulose nitrates for determining the viscosity of cellulose compounds.

The following study has been made to show that the cuprammonium method described by Ost (*19*) has many disadvantages and should be replaced by improved methods. It is known, especially through the research work of Traube (*23*) and Hess (*15*, *16*), that cuprammonium cellulose solutions or swellings contain an alcoholate in which the copper is bound partly as a cation and partly as an anion according to the formula:



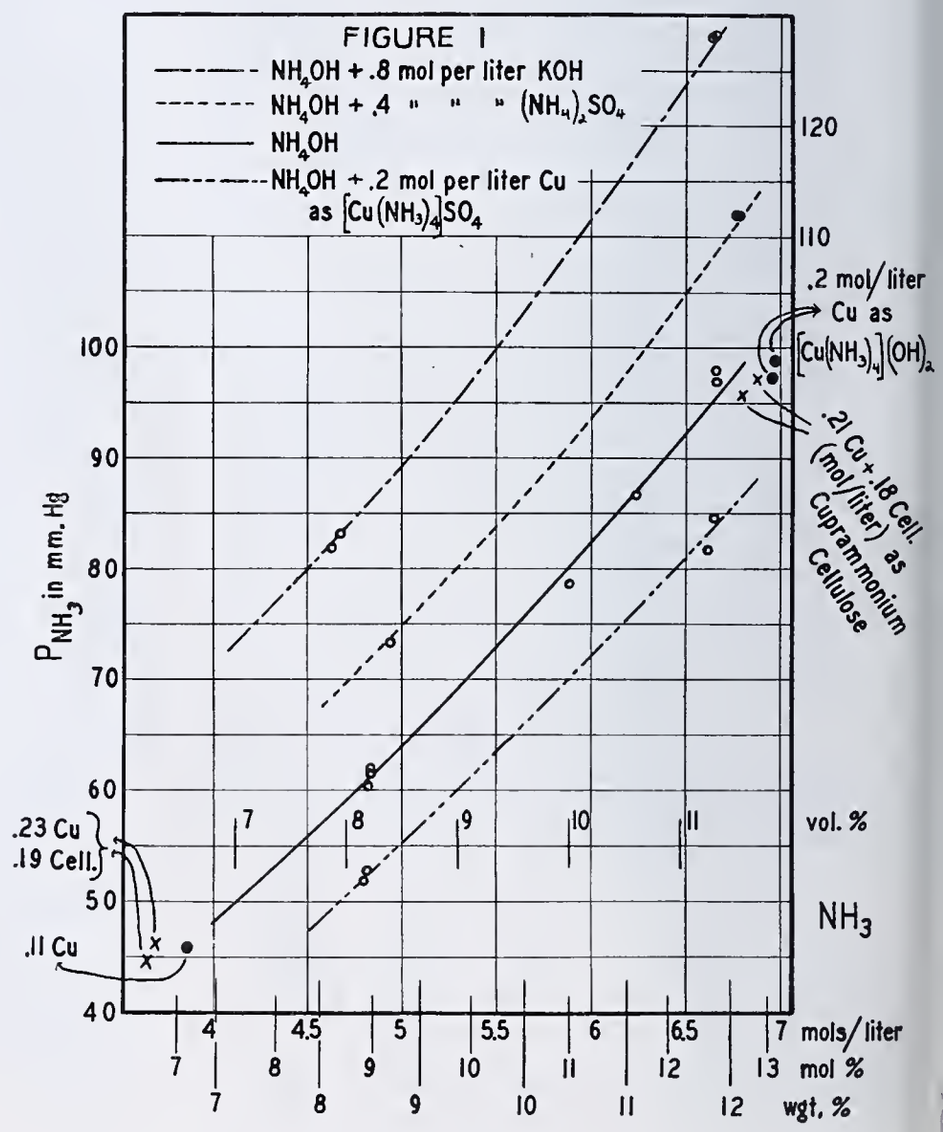
With his collaborator Innes (*7*), the author tried to get some insight into the composition of these copper cellulose solutions by determining the ammonia vapor pressure over cuprammonium and cuprammonium cellulose solutions. The results are reproduced in Figure 1.

Unfortunately on account of compensation phenomena—salting-out effects and stronger absorption of ammonia—it is impossible to determine in this way whether an increase in ammonia concentration really takes place when cellulose forms this alcoholate. It would necessarily occur if, according to Traube (*23*), 4 moles of ammonia are set free from the cuprammonium compound which furnishes the aniogen-bound copper atom. Observations in industry seem to show that by the dissolution of cellulose in cuprammonium a certain amount of ammonia is set free, so that the addition of more copper hydroxide and, therefore, of cellulose is made possible.

Figure 2 shows the different light absorption of solutions of copper tetrammino sulfate, cuprammonium, and the copper cellulose compound. The complex sulfate which does not dissolve cellulose has a different composition from the cuprammonium solution and the copper cellulose compound.

With Innes (*7*) and A. B. Cramer experiments were carried out which show how quickly cuprammonium cellulose solutions change their viscosity if they come in contact with oxygen. Oxygen is absorbed quickly, especially in the presence of mercury.

Figure 3 shows the apparatus which has been used.



Two Erlenmeyer flasks, wrapped in black paper to avoid the influence of light, were fixed on a noiseless shaking machine constructed by the author and his collaborators (*11*). The flasks contained the copper cellulose solution with 2.62 per cent of cellulose. In one flask some mercury was added. These flasks were connected with burets which allowed the measurement of quantity and speed of absorption of oxygen. Samples were taken from the flask and their viscosity was measured in an Ostwald viscometer.

Figure 4 shows the rate of absorption of oxygen by copper cellulose solutions and their change in viscosity with time. The solutions of cuprammonium cellulose absorb oxygen rather quickly. A quick reduction of the viscosity of the copper cellulose solution takes place. Combined with this oxygen absorption is a very strong degradation of the dissolved cellulose. Part of the oxygen is used to degrade and oxidize cellulose and its degradation products. Part of the oxygen transforms the ammonia into ammonium nitrite which is partly decomposed in alkaline medium with formation of free nitrogen, and partly oxidized with formation of ammonium nitrate.

In one experiment during the treatment of cuprammonium free of cellulose with oxygen 13.8 per cent of the ammonia was oxidized to ammonium nitrite according to the reaction: $2NH_3 + 1^1/_2O_2 = NH_4NO_2 + H_2O$. In this experiment 1137 ml. of oxygen were absorbed. The nitrogen present in the oxygen (about 4 per cent nitrogen) was taken into account. By the decomposition of ammonium nitrite 94.5 ml. of nitrogen were formed according to the reaction: $NH_4NO_2 = N_2 + 2H_2O$.

It can be seen from Figure 4 that the viscosity of cuprammonium cellulose solution is reduced to one half in the absence of mercury after 3.33 hours, and in the presence of

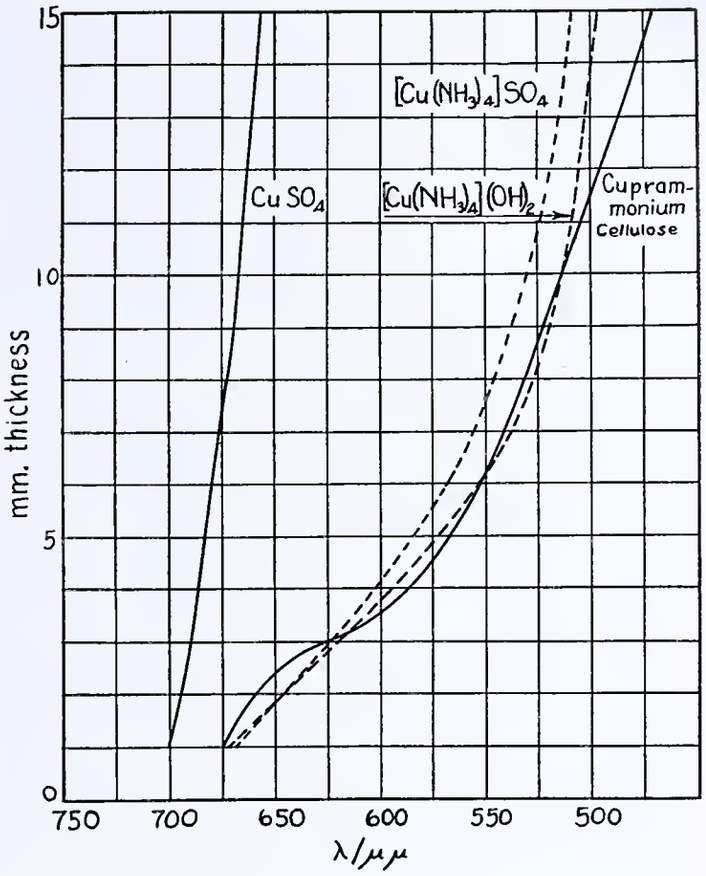


FIGURE 2. LIGHT ABSORPTION OF SOLUTIONS OF COPPER TETRAMMINO SULFATE, CUPRAMMONIUM, AND COPPER CELLULOSE COMPOUND

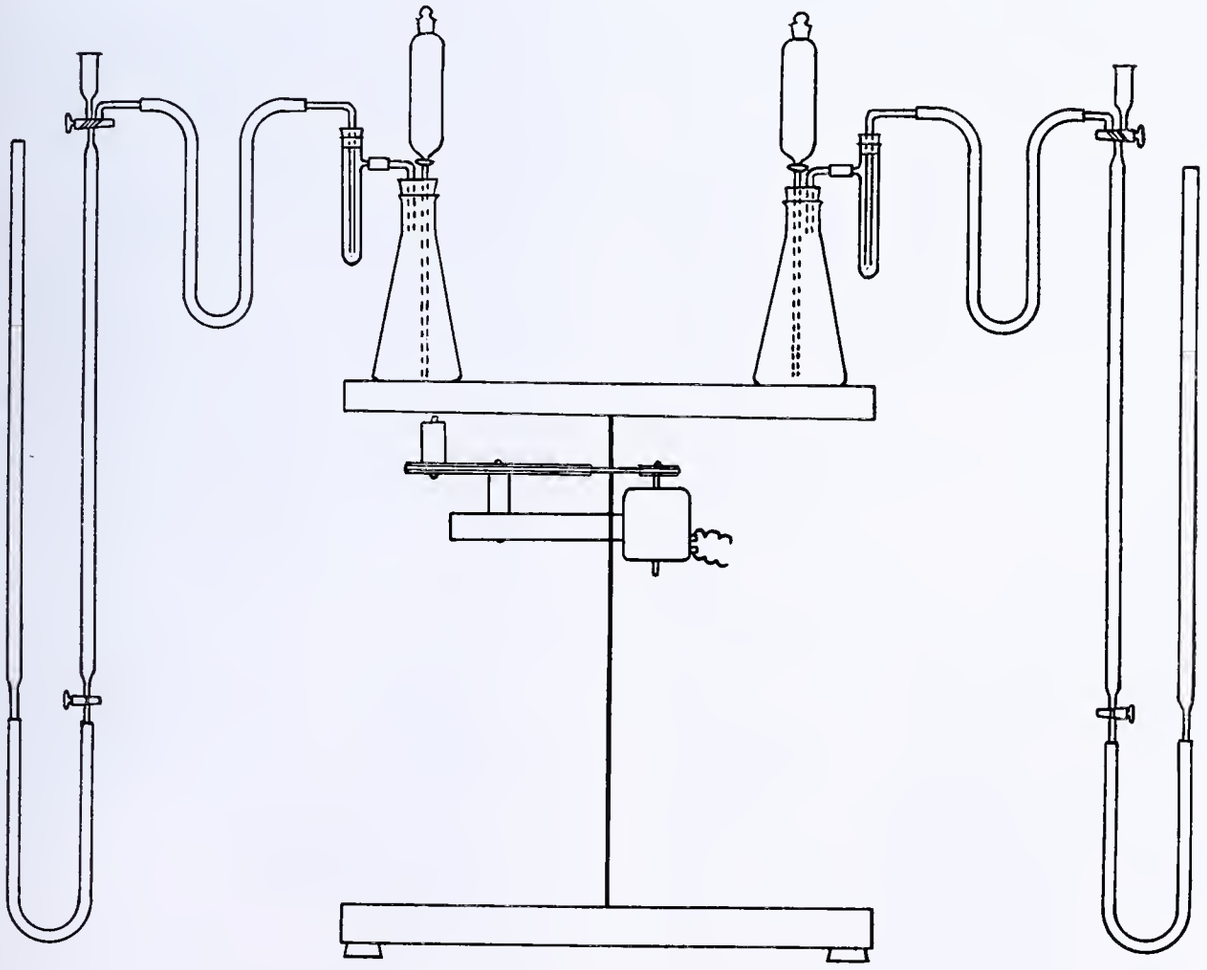


FIGURE 3. DIAGRAM OF APPARATUS

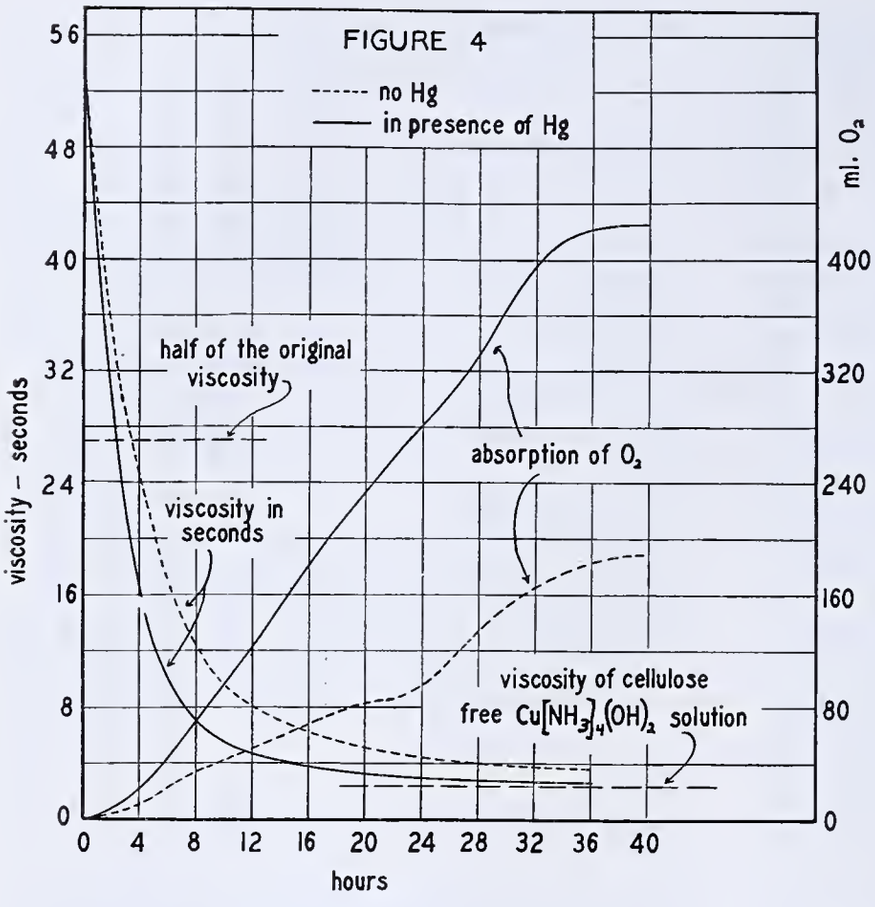


FIGURE 4
------ no Hg
—— in presence of Hg
half of the original viscosity
viscosity in seconds
absorption of O_2
viscosity of cellulose free $Cu[NH_3]_4(OH)_2$ solution
viscosity - seconds
ml. O_2
hours
0 4 8 12 16 20 24 28 32 36 40
0 8 16 24 32 40 48 56
0 80 160 240 320 400

mercury after 2.5 hours. After 24 to 36 hours the degradation of cellulose and the decrease in viscosity are so advanced that practically the viscosity of the cellulose-free cuprammonium solution (1.18 centipoises at 20.45° C.) is obtained. Those oxidized cellulose solutions upon acidification give only small amounts of a powdery precipitate. The original solution, not treated with oxygen, forms under the same conditions a much larger weight of a tough film.

We have to assume that in cuprammonium solutions most of the copper is bound in the form of a complex copper tetrammino hydroxide, but there may be a certain amount of colloidal copper hydroxide present, which probably acts as an oxygen carrier for the oxidation of cellulose and ammonia. Very strongly degraded oxycellulose with an acid character is formed. Small amounts of carbon dioxide are formed by this oxidation of the cellulose.

These experiments show what was known before—that the determination of viscosity by the copper method gives unreliable data unless the greatest care is taken. The strongly alkaline character of the copper ammonia complex causes degradation of cellulose in any case, even in the absence of oxygen. A fresh copper cellulose solution gave a viscosity of 155 seconds. After 3 days in a completely filled, closed, dark flask without action of oxygen a viscosity of only 130 seconds was observed (see also *19*). In the presence of oxygen strong degradation of cellulose took place. After several hours of oxidation a viscosity of 7 seconds was observed. Such results make this method rather unreliable.

The author with his collaborators, before and after the 1909 publication concerning the relationship between viscosity and micelle weight (*2*, *3*, *6*, *13*), studied the nitration of cellulose. With a mixed acid, composed of about 44 per cent sulfuric acid, 44 per cent nitric acid, and 12 per cent water, degradation of cellulose during nitration takes place, but this degradation is not very strong. It was found that nitrations with very little degradation can be carried out with a mixture of phosphoric and nitric acids containing several per cent of free phosphorus pentoxide—for instance, with mixtures containing 50 to 60 per cent of nitric acid, 25 to 35 per cent of phosphoric acid, and 5 to 15 per cent of phosphorus pentoxide. With Rueff (*10*) and W. L. C. Hui, the author has shown that such mixtures nitrate cellulose in less than 5 minutes to very high nitrogen contents, up to 13.8 to 13.9 per cent of nitrogen. Similar results can be obtained by a quick nitration of cellulose (lasting less than 5 minutes) with mixtures of water-free nitric acid and water-free glacial acetic acid containing 80 per cent nitric acid and 20 per cent acetic acid. Those experiments were carried out with Smith (*12*) and Ch. Carpenter. Nitrates with about 13.80 per cent nitrogen are obtained. Both mixed acids can be used at room temperature. After as much as possible of the adhering spent acid has been pressed from the nitrated material, this material is introduced into an excess of cooled (−10° to −30° C.), diluted alcohol (1 to 1), washed several times with this 45 to 50 per cent alcohol, boiled 3 times with 96 per cent alcohol for 5 minutes, and squeezed out after each boiling.

The whole operation—nitration and stabilization—takes about 45 minutes and forms highly nitrated, clear soluble cellulose nitrates of high stability. After stabilization the alcohol may be replaced by ether (free of acid and peroxides), which is removed in an evacuated apparatus at room or somewhat elevated temperature. This drying operation also is finished in a very short time, giving a somewhat hygroscopic dry material. Of this, 0.25 gram is dissolved either in 100 ml. of acetone (b. p. 56.5° C.), or better in 100 ml. of higher boiling *n*-butyl acetate (b. p. 125.1° C.), best in a glass flask which contains some glass balls, and then shaken during 1 or 2 hours on a shaking machine (*11*). The resulting homogeneous solution may be investigated in any viscometer, preferably in the one described by the author and Umstätter (*13*).

One gets stable cellulose nitrate solutions which after days of standing in carefully closed glass flasks with glass stoppers, or after having been investigated at higher temperatures, show the same viscosity at the same lower temperature. With Ch. Carpenter a rather large number of materials were investigated (Table I).

The raw materials of Table I are not intentionally degraded celluloses. They are commercial materials, but show rather great differences (50 to 1) in the relative viscosities of their carefully prepared nitrates. The values for un-

TABLE I. VISCOSITY OF CELLULOSE

Sample No.	Material	N %	η rel.	Log η rel.	Viscosity of 0.25% Nitrate Solution in *n*-Butyl Acetate *Centipoises*
1	Unbleached linters	13.76	173.8	2.24	134.4
2	Unbleached linters	13.79	158.5	2.20	122.5
3	Linters N-500	13.80	60.3	1.78	46.6
4	Ramie	13.86	37.2	1.57	28.75
5	Ramie	13.92	36.3	1.56	28.1
6	Brown wood pulp	13.70	26.9	1.43	20.8
7	Linters N-30	13.86	17.4	1.24	13.44
8	Pure cellulose wood pulp	13.72	17.4	1.24	13.44
9	Dynamite cellulose (nitrated with HNO_3-H_2SO_4-H_2O mixture)	12.3	15.2	1.18	11.75
10	Sulfite wood pulp	13.65	14.5	1.16	11.2
11	Linters 1000	13.84	12.8	1.11	9.9
12	Linters N-10	13.78	11.5	1.06	8.7
13	Guncotton (see No. 9)	13.6	10.0	1.00	7.73
14	Soda wood pulp	13.43	3.47	0.54	2.65

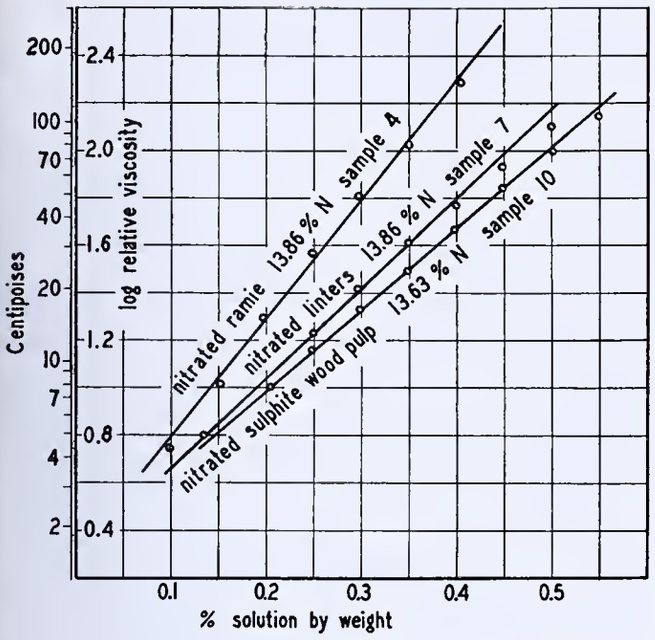

Centipoises
log relative viscosity
% solution by weight
nitrated ramie 13.86 % N sample 4
nitrated linters 13.86 % N sample 7
nitrated sulphite wood pulp 13.63 % N sample 10

FIGURE 5. RELATIONSHIP BETWEEN CONCENTRATION AND RELATIVE VISCOSITY OF CELLULOSE NITRATES PRODUCED BY PHOSPHORIC-NITRIC ACID NITRATION

t, 25° C.
Solvent, *n*-butyl acetate (0.773 centipoise)

TABLE II. VISCOSITY OF NITRATE SOLUTIONS

	Ramie		Linters	
	N %	η rel.	N %	η rel.
H_3PO_4, P_2O_5, HNO_3	13.9	37	13.8	13
CH_3COOH, HNO_3	13.6	34	13.6	13
H_2SO_4, H_2O, HNO_3	13.6	20	13.6	10

bleached linter nitrates are the highest ever obtained for a 0.25 per cent (0.25 gram of cellulose nitrate in 100 ml. of *n*-butyl acetate) solution in *n*-butyl acetate.

All these data were obtained by the nitration of different celluloses with mixtures of nitric acid, phosphoric acid, and phosphorus pentoxide with the exception of the dynamite cellulose (No. 9) and guncotton (No. 13) which were nitrated with nitric acid–sulfuric acid–water mixtures. The dynamite cellulose nitrates (No. 9) until now were considered as rather slightly degraded cellulose nitrates. The relative viscosity of their *n*-butyl acetate solution is less than 9 per cent of that of unbleached linter nitrates, resulting from the phosphoric acid (phosphorus pentoxide) nitration.

Figure 5 shows that over the range of concentration between 0.2 and 0.45 per cent and for viscosities between 8 and 80 centipoises the logarithmic Berl-Bütler relationship (*1*, *4*, *13*) between relative viscosity and concentration—$\log \eta_{rel.} = k \times C$—is valid. The data of Figure 5 concern samples 5, 7, and 9 of Table I (experiments carried out by Ch. Carpenter).

The relationship between micelle (molecular) weight and viscosity is not so simple as is described in the literature. The author has reason to believe that all derivatives of a nondegraded or very little degraded cellulose are insoluble and that a certain degradation is necessary to produce soluble cellulose derivatives. He has produced at low temperatures insoluble cellulose nitrates with nitrogen contents up to 13 per cent. Upon heating those insoluble esters with water in sealed glass tubes at 135° C., the nitrogen content decreases several tenths of a per cent and the resulting nitrates are acetone-soluble.

These experiments show that a small degradation (compared with one which occurs during nitration with sulfuric acid–nitric acid–water mixtures) results when cellulose of different origin and different treatment is nitrated with mixed phosphoric acid–nitric acid or with mixed glacial acetic acid–nitric acid. Table II shows the relative viscosity of nitrate solutions in *n*-butyl acetate (0.25 gram per 100 ml.).

About 3 years ago in Pittsburgh, the author had the privilege of discussing with The Svedberg of Upsala his important high-speed centrifuge. Professor Svedberg told him that with the help of his ultracentrifuge he found that viscose solutions during the first day show a reduction in micelle weight and that afterwards, until this viscose solution coagulates, the micelle weight remains constant.

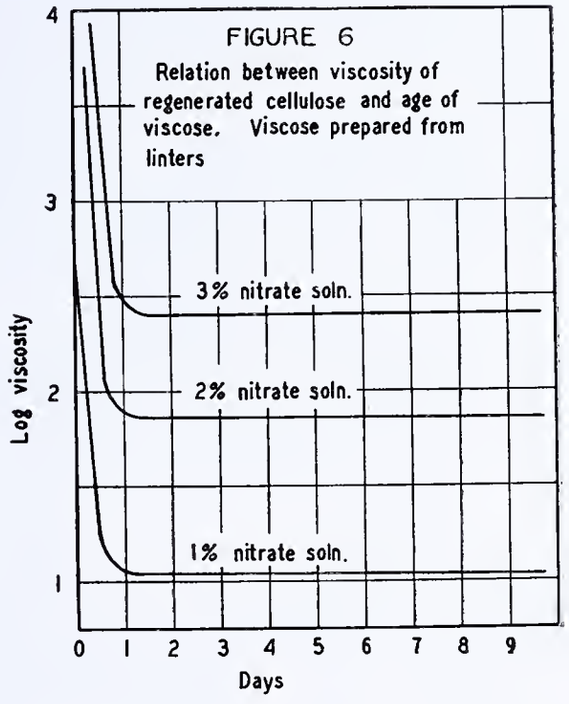


FIGURE 6
Relation between viscosity of regenerated cellulose and age of viscose. Viscose prepared from linters
3% nitrate soln.
2% nitrate soln.
1% nitrate soln.
Log viscosity
Days

Fifteen years ago with Lange the author (*9*) studied viscose. After different time intervals carefully coagulated samples were dried and nitrated, and the viscosity of those nitrates was determined. The results (Figure 6) are in complete agreement with Svedberg's ultracentrifuge observations. We know (*5*) that viscose solutions show first a decrease and then an increase in viscosity (Figure 7). This increase in viscosity has nothing to do with an increased micelle weight, as can be seen from the author's experiment, but is due to

FIGURE 7. RELATION BETWEEN VISCOSITY AND AGE OF VISCOSE

the formation of a gel structure. The decrease in viscosity is a function of degradation and desolvation.

These newer nitration methods have the great advantage that they can be carried out very quickly. They give nitrates which in repeated experiments show the same viscosities of their solutions if the same cellulosic material and the same conditions of nitration are used.

Nitration, washing, drying, and dissolution of nitrates are rather harmless operations which can be carried out in every laboratory by the trained chemist and workman. The stabilized nitrates do not change their properties if kept wet in carefully closed containers. When no longer needed the nitrated material may be burned in small quantities, or decomposed by putting it into warm, strong alkali.

The copper method is a very doubtful one. Like other strong alkalies (*2*), the strong alkaline cuprammonium degrades cellulose to a certain extent in the absence of oxygen. In the presence of oxygen a very strong degradation of cellulose takes place with absorption of rather large quantities of oxygen. The cellulose is transformed into oxidized, strongly degraded compounds with low viscosity. The copper method requires a much longer time than the newer nitration methods.

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Presented before the Division of Cellulose Chemistry at the 100th Meeting of the American Society, Detroit, Mich.

Determination of Mercaptan Sulfur Content of Gasolines and Naphthas

Effect of Mercuric Sulfide and Elementary Sulfur

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THE mercaptan sulfur content of gasoline or naphtha is usually determined by titration using silver nitrate (*3*), ammonium thiocyanate, and ferric alum indicator. When elementary sulfur is present in the sample it is common practice to remove it by shaking the gasoline or naphtha with metallic mercury and filtering off the mercuric sulfide formed before titration of the gasoline or naphtha with the silver nitrate. Borgstrom and Reid (*1*) investigated the effect of metallic mercury, but not mercuric sulfide, on the mercaptan determination and found no substantial decrease in the value for per cent mercaptan sulfur after the mercury treatment. These experiments, however, were carried out in the absence of free sulfur. In practice, free sulfur is not infrequently present. The authors have found that the results of mercaptan sulfur determinations regularly are too low when the elementary sulfur present in the gasoline has been removed as mercuric sulfide. They have found, furthermore, that the mercaptan sulfur content of gasoline or naphtha can be quantitatively determined by the Borgstrom and Reid method (*1*) without previous removal of any elementary sulfur which may be present.

Tests were carried out on solutions of the following mercaptans in Stoddard solvent: ethyl mercaptan, *n*-butyl mercaptan, *n*-propyl mercaptan, *n*-amyl mercaptan, *n*-heptyl mercaptan, benzyl mercaptan, isobutyl mercaptan, and *tert*-butyl mercaptan. A blend of equal parts of the solutions of ethyl mercaptan, *n*-propyl mercaptan, *n*-amyl mercaptan, and isoamyl mercaptan was also used in the tests. The solutions thus prepared contained 0.04 to 0.07 per cent of mercaptan sulfur. When it was desired to have elementary sulfur present in the test solution, Stoddard solvent containing 0.158 per cent of elementary sulfur was blended with the mercaptan solution.

The effect of the removal of elementary sulfur, as mercuric sulfide, on the mercaptan sulfur content of the Stoddard solvent is shown in Table I. The elementary sulfur was removed by shaking 50 cc. of the test solution with 2 cc. of metallic mercury in a 120-ml. (4-ounce) bottle for 15 minutes. The mercuric sulfide formed was removed by filtration through Whatman No. 40 filter paper.

Table I illustrates the decrease in mercaptan sulfur found in the Stoddard solvent after removal of the elementary sulfur as mercuric sulfide. It also indicates that the decrease in mercaptan sulfur content is dependent upon the particular mercaptan present and decreases with increase in the length of the hydrocarbon chain attached to the mercaptan group. Tertiary butyl mercaptan is not influenced by the mercuric sulfide.

Table I. Effect of Removal of Elementary Sulfur by Metallic Mercury on Mercaptan Sulfur Content of Stoddard Solvent

Mercaptan	Elementary Sulfur in Test Solution	Mercaptan Sulfur Found	Mercaptan Sulfur Initially present	Change in Mercaptan Sulfur Content Due to Shaking with Metallic Mercury
	% by weight	%	%	%
Ethyl	0.006	0.060	0.075	−20
n-Propyl	0.026	0.051	0.055	− 7
n-Propyl	0.059	0.036	0.041	−12
n-Butyl	0.026	0.046	0.054	−15
n-Butyl	0.059	0.036	0.041	−12
n-Amyl	0.026	0 057	0.056	+ 2
n-Amyl	0.059	0.042	0.041	+ 2
n-Heptyl	0.059	0.039	0.039	0
Benzyl	0.026	0.054	0.053	+ 2
Benzyl	0.059	0.037	0.040	− 8
Isobutyl	0.026	0.052	0.058	−10
Isobutyl	0.059	0.039	0.043	− 9
tert-Butyl	0.059	0.043	0.043	0
Blend of equal parts of ethyl, *n*-propyl, *n*-amyl, and isoamyl mercaptan solutions	0.008	0.037	0.040	− 8

The decrease in the mercaptan sulfur content of the Stoddard solvent appears to be due to adsorption of mercaptans by the mercuric sulfide formed from the interaction of free sulfur and metallic mercury. This postulate of adsorption was tested in the following manner:

Mercuric sulfide, which had been precipitated from aqueous mercuric chloride solution by hydrogen sulfide and was therefore free from mercaptans, was collected on a Büchner funnel, washed twice with water, with 95 per cent alcohol, and then with naphtha, after which it was shaken with naphtha containing 0.05 per cent mercaptan sulfur (present as *n*-propyl mercaptan). The mercuric sulfide was collected on a Büchner funnel, then shaken with mercaptan-free naphtha, which acquired sufficient mercaptan to give a positive mercaptan test. The presence of mercaptan was found even in a third treatment of this mercuric sulfide with fresh mercaptan-free naphtha.

These results show that some of the mercaptan which had been taken up by the mercuric sulfide was extracted by the fresh naphtha and indicates that *n*-propyl mercaptan is adsorbed by mercuric sulfide. That metallic mercury in the absence of elementary sulfur does not react with mercaptans is well known and has been confirmed by the results shown in Table II.

Any factor which favors rapid formation of mercuric sulfide, such as increasing the amount of mercury used in treatment of the gasoline or increasing the amount of sulfur in the gasoline, increases the removal of mercaptan sulfur from the Stoddard solvent. Furthermore, the authors have found that the addition of mercuric sulfide to a mercaptan solution decreased the percentage of mercaptan sulfur as found by silver nitrate titration. This is illustrated in Tables I, III, and IV.

Table II. Effect of Metallic Mercury

(On determination of mercaptan sulfur content of Stoddard solvent where no elementary sulfur is present)

Mercaptan	Mercaptan Sulfur Found (after shaking with metallic mercury)	Mercaptan Sulfur Initially present	Deviation
	%	%	%
Ethyl	0.078	0.078	0
Mercaptan blend (see Table I)	0.040	0.040	0

Table III. Effect of Amount of Mercury and Elementary Sulfur

(On determination of mercaptan sulfur content of Stoddard solvent)

Metallic Mercury Added to Mercaptan Blend (See Table I)	Elementary Sulfur in Solution	Mercaptan Sulfur Found	Mercaptan Sulfur Initially present	Change from Mercaptan Sulfur Initially Present
Cc.	%	%	%	%
0	...	0.040	0.040	0
1	0.008	0.038	0.040	− 5
3	0.008	0.037	0.040	− 8
6	0.008	0.035	0.040	−13
3	0.014	0.030	0.034	−12

Table IV. Treatment of Mercaptan–Stoddard Solvent Blend with 1 Per Cent of Mercuric Sulfide

Mercaptan Sulfur Found	Mercaptan Sulfur Initially present	Change from Mercaptan Sulfur Initially Present
%	%	%
0.042	0.049	−14

Table V. Effects of Various Conditions

(On determination of mercaptan sulfur content of Stoddard solvent)

Mercaptan	Condition Used	Mercaptan Sulfur Found	Mercaptan Sulfur Initially present	Change from Mercaptan Sulfur Initially Present
		%	%	%
Ethyl	Agitation with air	0.077	0.078	−1
Ethyl	Agitation with filter paper	0.079	0.078	+1
Ethyl	Filtration through filter paper	0.076	0.078	−3
n-Butyl	Agitation with filter paper	0.064	0.065	−2
n-Butyl	Filtration through filter paper	0.065	0.065	0

Table VI. Effect of Elementary Sulfur

(On determination of mercaptan sulfur content of Stoddard solvent by titration with silver nitrate)

Mercaptan	Elementary Sulfur by Weight	Mercaptan Sulfur Found	Mercaptan Sulfur Present	Error on Basis of Mercaptan Sulfur Actually Present
	%	%	%	%
Ethyl	0.060	0.075	0.075	0
n-Butyl	0.026	0.055	0.054	+2
n-Propyl	0.059	0.042	0.041	+2
n-Amyl	0.059	0.042	0.041	+2
n-Heptyl	0.059	0.039	0.039	0
Benzyl	0.059	0.041	0.041	0
Isobutyl	0.026	0.059	0.058	+2
Isobutyl	0.059	0.045	0.043	+5
tert-Butyl	0.059	0.044	0.043	+2
Mixture of ethyl, *n* - propyl, *n*-amyl, and isoamyl mercaptans	0.008	0.040	0.040	0

That adsorption of mercaptans on metal sulfides takes place to a considerable extent has been found by Greer (*2*), who used cupric sulfide to remove mercaptans from gasoline and thus to sweeten it. Lead, stannic, cadmium, and arsenious sulfides were also found to be effective, but to a lesser degree than cupric sulfide; mercuric sulfide was not tested by Greer.

In order to be certain that adsorption on mercuric sulfide caused the decrease in the percentage of mercaptan sulfur as determined by silver nitrate titration after the removal of free sulfur, the authors investigated other factors that might be responsible for this lowering effect, such as the loss of mercaptans by evaporation during filtration of the mercuric sulfide from the Stoddard solvent, the possibility of adsorption of mercaptans by the filter paper used [tested by shaking 50 cc. of the mercaptan solution in a 120-ml. (4-ounce) bottle with strips of Whatman No. 40 filter paper], and possible oxidation by the oxygen of the air [tested by shaking the solution in a 120-ml. (4-ounce) bottle for 15 minutes]. The results obtained are shown in Table V.

All the results in Table V are within the limits of experimental error of the mercaptan sulfur determination, thus showing that agitation with air or filtration through Whatman No. 40 filter paper is without appreciable effect on the determination.

Since the authors' results indicated that there was a noticeable difference between the amount of mercaptan sulfur found after treatment of the Stoddard solvent containing elementary sulfur with metallic mercury and that amount which is actually present in the material, it is advisable not to remove the free sulfur prior to the mercaptan sulfur determination but to titrate the mercaptan sulfur present in the Stoddard solvent directly with silver nitrate. That the elementary sulfur has no effect on the determination of mercaptan sulfur in the Stoddard solvent is shown by Table VI.

The deviations from the amounts of mercaptan sulfur actually present as shown in Table VI are all within the limits of experimental error, showing that it is justifiable to determine the mercaptan sulfur content of Stoddard solvent (or other hydrocarbon material) by titration with silver nitrate without removal of elementary sulfur present in the material under test.

Where a considerable quantity of elementary sulfur is present, the color of the precipitate obtained during titration of the mercaptans with silver nitrate solution may pass through various shades of orange, red, or brown and persistent emulsions may be formed. The addition of quantities of 95 per cent alcohol as high as 100 cc. may be necessary to break these emulsions and bring the precipitate into the interface so that the color of the solution may be noted.

Summary and Conclusions

Contrary to the current practice of removing elementary sulfur by means of metallic mercury before determination of the mercaptan sulfur content of gasolines or naphthas, the determination of the mercaptan sulfur content should be made on the initial gasoline or naphtha, ignoring any elementary sulfur which may be present. Certain mercaptans are adsorbed by mercuric sulfide, resulting in such cases in low values for the mercaptan sulfur content of the naphtha which has been agitated with mercuric sulfide. Any loss of mercaptans which might take place on filtration of Stoddard solvent solutions through Whatman No. 40 filter paper or on normal agitation of such solutions with air is within the limits of experimental error of the mercaptan sulfur determination.

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Determination of Water in Benzene

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A solution of sodium triphenylmethyl in ethyl ether has been investigated as a reagent for the analysis of water in solution in benzene. The analyses have been made on samples containing a relatively small concentration of water. The color change of the reagent is used as the indicator, and relatively high precision is obtainable on low water concentrations. The reagent is suitable for the analysis of other substances.

A NUMBER of ingenious methods have been devised for the analysis of water dissolved in benzene and other organic liquids—for example, the use of calcium carbide (*3*) with the determination of the acetylene produced; the use of calcium hydride (*8*) with the determination of the evolved hydrogen; the use of alpha-naphthoxydichlorophosphine (*1*, *7*) with titration of the hydrogen chloride; the use of a reagent consisting of liquid sulfur dioxide and iodine dissolved in anhydrous methyl alcohol (*4*), where the reagent reacts with water forming sulfuric and hydroiodic acids, which are caused to combine with pyridine, and the excess iodine is determined; and the determination of the solubility of silver perchlorate (*6*) which varies with the water content of benzene.

The authors have used a solution of sodium triphenylmethyl dissolved in ethyl ether for the determination of water in solution in benzene. Bent and Lesnick (*2*), who used this reagent to determine the amount of water adsorbed on glass surfaces, indicate that the partial pressure of water in equilibrium with this substance is very low and that equilibrium is reached rapidly. In order to obtain samples of benzene containing reproducible concentrations of water, a stream of nitrogen containing a known partial pressure of water was passed through dry benzene. The titration with the standardized reagent was accomplished without removing the sample from the apparatus. The standardization was also done within the apparatus by means of a sample of moist nitrogen.

Apparatus

The apparatus used is shown in Figure 1.

Tube 46 is the entry tube for dry oxygen-free nitrogen, for which three paths of travel are provided. For use in operating valves, siphons, etc., it passes through three phosphorus pentoxide drying tubes, 13, 14, and 15, each 60 cm. long and about 2.5 cm. in diameter, and then enters manifold 41. For use as a carrier gas for water vapor at its saturation pressure at 25° C. it passes through drying tube 12 and then through bubblers 1, 2, and 3 (only one shown); at other temperatures, it passes through drying tube 16 and then bubblers 4, 5, 6, 7, 8, and 9 (only one shown).

Vessels 10 and 11 are bubblers for observing the rate of gas flow. Vessels 17, 20, 21, 24, and 28 are valves containing mercury which are opened by suction or closed by nitrogen pressure from auxiliary manifold 43, vessel 38 being the mercury storage for 28. Tube 18 is the inner tube of valve 17. Tube 35 is for equalizing pressure. Vessels 47, 34, 36, 49, and 27 are mercury bubblers designed to protect stopcocks from ether and benzene vapors. Vessel 48 is a mercury bubbler to prevent water vapor from vessels 4 to 9 coming in contact with the benzene solution. In vessel 19, which has a volume of about 200 cc., water is dissolved in benzene by passing moist nitrogen through it. Mercury valve 21 serves to remove a sample from 19 and place it in the titrating vessel, 22. Storage vessel 30 with a volume of 350 cc. is for supplying the ether solution of sodium triphenylmethyl the discharge of which is through tube 31.

Buret 29, which has a volume of 22 cc., and is calibrated in 0.25 cc. divisions, contains a glass plunger, 39, which is ground to fit the lower opening of the buret. A pure gum rubber tubing gives a flexible connection at the top for operation of the plunger. The reagent flows through tube 45 and its capillary tip. Vessel 22, the titrating vessel, has the same dimensions as the buret and is calibrated in 1-cc. divisions. Tip 40 is used in the clean-

ing operations. Vessel 23 is a gas buret of 250-cc. volume calibrated in 5-cc. divisions, and connected to mercury storage vessel 42. Capillary tubes 26, 25, and 37 provide outlets to the atmosphere while preventing back-diffusion into the apparatus. Additional protection against back-diffusion of the atmosphere into the apparatus is provided by the connections which enable a stream of dry nitrogen to pass from manifold 41 through these outlets.

Vessels 1, 2, 3, 17, 19, 20, 21, 22, 23, and 24 with their connecting tubes were kept in a water thermostat at 25° C. It was found necessary to have two small sections of connecting tubing above the water level of the thermostat, and these were wound with resistance wire and heated to prevent the condensation of water in them.

Preparation of Materials

Nitrogen was taken from a commercial cylinder and was passed through both a tube containing copper turnings heated to 420° C. and sulfuric acid bubblers.

Thiophene-free benzene was dried differently for the different experiments (Table I). For experiments A_1 and A_2 it was allowed to stand over freshly cut sodium for 2 weeks. For experiments C_1, C_2, C_3, and C_4 it stood over a large quantity of phosphorus pentoxide for 2 weeks and was shaken frequently.

For experiments E_1 to E_5, in which it was considered anhydrous, it was dried by passing the vapor slowly through phosphorus pentoxide. The apparatus for this consisted of two vertical cylindrical vessels connected by a horizontal tube wound externally with a heating coil. Both vessels and tube contained phosphorus pentoxide and each vessel could be heated. Eleven passages of the material were performed before the benzene was considered anhydrous and was transferred to the analysis apparatus. In experiments D_1 to D_4 the residual benzene from experiments E_1 to E_5 was used, and in experiments B_1 to B_4 the residual benzene from experiments D_1 to D_4 was used. In all cases the benzene was boiled to remove dissolved oxygen before drying and also before introduction into the apparatus.

Sodium triphenylmethyl solution in ethyl ether was prepared in a 200-cc. cylindrical vessel, the top of which terminated in a small-bore tube. To the side another tube was connected which terminated in a long capillary sealed at the lower end. With the vessel dry and containing oxygen-free nitrogen 175 cc. of anhydrous ether were added, followed by 35 to 45 grams of 28 per cent by weight sodium amalgam and 9 grams of triphenylchloromethane. The top tube was sealed and the vessel vigorously shaken for 5 minutes about every hour for 2 days.

Procedures

The sodium triphenylmethyl solution was introduced into storage vessel 30 through tip 32. The vessel was dried by passing purified nitrogen through it for 12 hours, tip 32 was then broken, a slow stream of nitrogen was allowed to exhaust through it, and the capillary tip of the vessel containing the reagent was opened and inserted in 32. One of the other connecting tubes of the reagent vessel was connected to a source of dry nitrogen by means of rubber tubing, and the reagent was slowly forced into 30. Tip 32 was then sealed.

The benzene was introduced in a similar manner into vessel 19 through a side tube on its top (not shown in Figure 1). The entire apparatus was evacuated before operations were started. After all vessels, connecting tubes, etc., had been dried they were filled with dry nitrogen.

The technique of operation consists of passing purified nitrogen from the manifold first through saturators and then through benzene in vessel 19. A sample is withdrawn from 19 and placed in vessel 21 by operating the appropriate mercury valves through the use of both nitrogen pressure and vacuum, and enough is forced into titrating vessel 22 for analysis. The reagent is forced into 29 in a similar manner and the titration is conducted by plunger 39. Stirring is accomplished during a titration by means of occasional passage of nitrogen from 44 through 22. The end point is judged from the first appearance of a permanent faint pink tint in the benzene.

In order to make sure that the titrating vessel is completely dry before a titration is begun, a quantity of reagent is dropped into it, allowed to stand for a few hours, and then forced out through tip 33. After a sample is titrated it is forced out through 33, and a new sample is taken from 21 for a check analysis. To establish the attainment of equilibrium this operation is repeated with further passages of wet nitrogen through the benzene. It was found that the rate of passage of the nitrogen below 5 cc. per minute had no effect on the results.

In order to standardize the reagent a known volume was placed in the titrating vessel, and nitrogen from vessel 23 containing a known partial pressure of water was slowly bubbled through it, until it just became discolored.

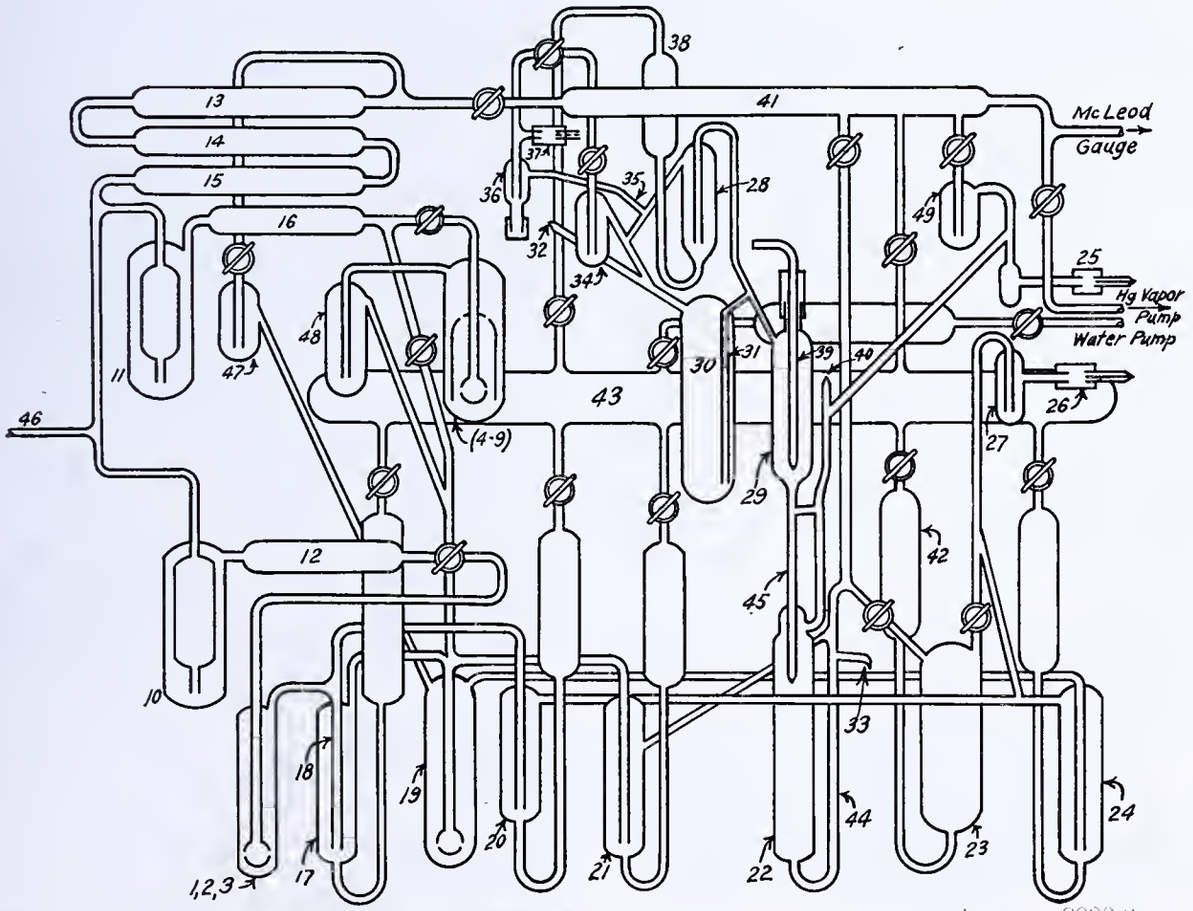

Figure 1. Apparatus Used for Determining Water in Benzene

Table I. Analysis of Water in Benzene at 25°

Experiment No.	Rate of Flow of Nitrogen	Time of Flow	Analysis		Standardization			Water in Benzene		Partial Pressures of Water	
			Volume of benzene	Volume of reagent	Volume of wet nitrogen	Volume of reagent	Water equivalent of reagent	Titration	Corrected average	Entering nitrogen	Leaving nitrogen
	Cc./min.	*Hours*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*G./cc. × 10⁻⁴*	*% by weight*		*Mm. of Hg*	
A_1	...	..	7.80	17.20	75	6.00	2.845	0.0709	...	23.517	25.51
A_2	...	...	7.80	17.27	90	7.15	2.868	0.0711	...	....	...
					93	7.23	2.822	....	0.067	....	....
B_1	2.1	48	12.04	4.27	400	10.0	9.114	0.0362	...	12.00	10.48
B_2	...	..	12.04	4.31	405	10.0	9.233	0.0365	...	....	...
B_3	...	..	12.04	4.26	...	...	....	0.0360	...	....	...
B_4	1.7	24	13.10	4.58	...	...	....	0.0357	0.032	....	...
C_1	2.3	52	12.04	1.63	347.5	7.23	10.12	0.0165	...	4.57	3.99
C_2	1.8	32	15.13	3.96	183	7.25	5.760	0.0168	...	....	...
C_3	...	..	15.50	4.05	181	7.25	5.712	0.0168	...	....	...
C_4	1.7	48	15.13	3.94	...	...	....	0.0167	...	....	...
C_5	...	..	15.20	3.99	...	...	....	0.0169	0.013	....	...
D_1	1.8	48	14.68	0.93	468	10.00	10.67	0.0076	...	1.08	0.94
D_2	...	..	15.28	1.00	473	10.00	10.73	0.0078	...	....	...
D_3	1.7	24	15.28	0.97	...	...	....	0.0078	...	....	...
D_4	...	..	15.28	1.00	...	...	....	0.0079	0.004	....	...
E_1	0	0	11.51	0.40	450	9.50	10.80	0.0042	...	....	...
E_2	...	..	23.00	0.70	473	10.00	10.82	0.0037	...	....	...
E_3	1.8	52	11.51	0.38	...	...	....	0.0039	...	6×10^{-4}	...
E_4	...	..	15.63	0.40	...	...	....	0.0031	...	....	...
E_5	...	..	15.63	0.45	...	...	....	0.0035	0.000	....	...
F	0	0	8.33	1.60	....	...	....	0.0233	0.019	....	...

Corrections, determined in two parts, were applied in the benzene titrations for the amount of reagent that would just cause the faint pink color used as the end point. First, to a sample of benzene in the titrating vessel enough reagent was added to cause a faint pink color. The sample was observed until the color had just disappeared. The amount of reagent needed to bring the color back to the shade used in the determinations was the correction applied to every titration. Because the visual observation of the disappearance of the pink color may be in error or a trace of reactive impurity other than water may be present in the benzene, a second correction was applied that is equal to the minimum value of the titration using the best drying technique. Its value was 0.004 per cent by weight.

The pure nitrogen or the exhaust from the saturators could be analyzed at any time by taking a sample in vessel 23. The purified nitrogen, when passed through a dilute sample of the reagent in the titrating vessel, completely evaporated the ether without decolorizing it. From this result it may be calculated that the partial pressures of oxygen, water, or other reactive impurities are less than an effective partial pressure of water of 1×10^{-4} mm. of mercury.

A mixture of equal volumes of acetone and water was found satisfactory for cleaning the titration and other vessels.

For experiments A_1 and A_2 the benzene in vessel 19 was mixed with water until it contained a liquid water phase. It was stirred by means of nitrogen, which was saturated with water at 25° C. by passing through bubblers 1, 2, and 3 containing freshly boiled distilled water. For experiments B_1 to B_4 the nitrogen was bubbled through vessels 4, 5, 6, 7, 8, and 9 which contained an aqueous solution of calcium nitrate of sufficient concentration to give a partial pressure of water of 12 mm. of mercury at 25°. In experiments C_1 to C_5 the nitrogen was bubbled through pure water in vessels 4, 5, 6, 7, 8, and 9 kept at 0° in ice. In experiments D_1 to D_4 the nitrogen was bubbled through the same vessels, containing an aqueous solution of potassium hydroxide of a concentration sufficient to give a partial pressure of water equal to 1.08 mm. of mercury at 0° C. In experiments E_1 to E_5 the nitrogen passed through traps immersed in a stirred carbon dioxide–acetone bath. The partial pressure of water in this nitrogen is 6×10^{-4} mm. of mercury or less. The partial pressure of water in the nitrogen escaping from the benzene solution was calculated, taking into consideration the partial pressure of benzene, the amount of water evaporated with the benzene from the solution, the barometric pressure, etc.

Rigorous precautions were taken to exclude oxygen from the apparatus and to exclude water from those portions where its presence was not desired. It was found that the reagent could be left for months in the apparatus without decolorization. Despite the fact that the titrating vessel was washed with the reagent before a determination was made, a calculation was made (*5*) of the amount of water that could be obtained from the glass surface. It was found that the maximum amount of water that could be added to the benzene from this source was about one twentieth the second part of the correction or 0.0002 per cent.

Results

In Table I are shown the results of the determination of the water content of benzene using a solution of sodium triphenylmethyl as the reagent. Experiments A_1 and A_2 give the values for benzene in contact with liquid water and saturated with it at 25° C. Experiments E_1 to E_5 give the second correction term. E_1 and E_2 were determined on benzene that had been thoroughly dried in the vapor phase, and E_3, E_4, and E_5 were checks made after nitrogen, which had been dried by passage through traps at −80° C., had been bubbled through the benzene. Experiment F was a single determination on a sample of benzene dried by standing 2 weeks over sodium and 2 weeks over phosphorus pentoxide with intermittent stirring. This shows that the liquid in contact with a drying agent is dried very slowly even with stirring. The determinations and standardizations bracketed together in the table are duplicates on the same sample. In experiments B, C, and D subsequent sets of determinations are for the benzene sample that has had wet nitrogen passed through it for an additional period of time.

The only determinations of the solubility of water in benzene at this temperature that have been published are for the two-liquid-phase saturated system. In per cent by weight these are 0.072 (Hill, *6*), 0.070 (interpolated from solubility values given at various temperatures by Groschuff, *5*), and 0.0652 (interpolated from curve of Rosenbaum and Walton, *8*). The authors found a value of 0.067.

The results herein reported show that a solution of sodium triphenylmethyl in ethyl ether is a very satisfactory reagent for the analysis of small amounts of water in an organic liquid. It is extremely sensitive and has the advantage of serving as its own color indicator. As this reagent will also react with many other substances such as oxygen, hydrogen chloride, etc., it can be used for the determination of these substances. Because of this, only one reactive substance should be present in the sample or, if more than one is present, all but one should be determined or removed by other means.

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Measuring Smokes and Rating Efficiencies of Industrial Air Filters

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To attain the extremely clean air required by some industrial processes, the ultimate in air filtration is necessary. A technique is described whereby it is possible to classify air filters critically by continuous measurement of concentrations of smokelike materials in filtered and unfiltered air. By determining density differences of impingement traces, it is possible to calculate percentage efficiencies of air filters. The apparatus consists of an impingement recorder and a photoelectric densitometer. The recorder operates continuously by impingement of air from a self-metering orifice on moving paper. The unit is readily portable.

MANY modern industrial processes must be conducted in an extremely clean atmosphere, so that the product being manufactured will remain uncontaminated or the equipment being used will continue to function well. Examples of such requirements are to be found in the photographic, optical, and communication industries. For these purposes, the air cleaned by the ordinary methods of filtration is not completely satisfactory—for example, viscous-type air filters, consisting of a liquid or plastic film spread on a surface of large area, are effective in removing large particles. In terms of percentage removal by weight the efficiency may be high, yet the amount of fine material which they allow to pass is appreciable.

Very finely divided air-borne particles will agglomerate in time to form larger aggregates. This effect has been studied scientifically by Whytelaw-Gray and Patterson (*12*). Practically, it is noticed that ducts fed with filtered air will in time become coated with particles of agglomerated finer material, which become a source of much trouble when accidentally dislodged. It is in an attempt to diminish this source of trouble that more effective methods of air filtration have been studied recently in this laboratory. For this study it was found necessary to develop a new technique which has proved sufficiently useful to merit description.

This technique of rating air filters consists of three general steps: (1) impingement of dirt on moving paper, (2) reading of the densities of the recorded traces, and (3) evaluation of filters by comparing the densities of the traces.

The impingement is accomplished by continuously drawing the air being investigated through a jet, and thence to the surface of a paper chart, at an extremely high and uniform velocity. The dirt in the air is thus transferred to the paper chart which is being moved slowly below the jet. The reading of the recorded traces can be accomplished by comparison with known standards or by means of a densitometer. Filter efficiencies are determined by operating impinging equipment simultaneously in filtered and in unfiltered air and then making calculations based upon the density differences shown by the two recorder traces.

Impingement Apparatus

The vital point of the design of the Kodak smoke recorder is in the manner in which the jet is utilized. As long as the pressure drop across the jet exceeds 0.53 atmosphere the flow of air is constant (*11*). By the use of this principle, one avoids in a simple manner the question of metering of air, which has proved an almost unsurmountable obstacle in the design of similar recorders by other workers in the past.

The orifice of the jet used for quantitative studies is rectangular in shape and about 0.178 × 1.07 mm. (0.007 × 0.042 inch) in size. It permits 2.15 liters (0.076 cubic foot) per minute of air to impinge upon a disk of 11.0-cm. Whatman No. 50 filter paper held and driven by a 24-hour clock of the kind used for recorders of the usual industrial type. The jet is held rigidly in such a manner that the long dimension of the rectangular opening lies on a radius of the chart and the end of the jet is about 0.63 mm. (0.025 inch) above the paper. At distances greater or less than this, the traces have indefinite edges, while at about 0.63 mm. (0.025 inch) traces with sharp edges are obtained.

Figures 1 and 2 show a favored jet construction. The tapered portion of the body next to the cut that receives the "slab" is relieved eccentrically, so that the sleeve, when it is driven into position, will be unhampered in squeezing the slab against its seating surfaces. The sleeve generally need not be driven more than 0.15 cm. (0.06 inch). If the slab does not fit perfectly against the body, it may be necessary to fill the open ring between the body and the sleeve with solder. It is important that the bell-mouth entrances to the orifices be smooth and of the same shape, since the coefficient of discharge is dependent upon the condition of the entrance to the orifice and variations in shape influence the output of jets otherwise closely similar.

The jet dimensions mentioned above were chosen to pass a volume of air of such order of magnitude as to be handled by laboratory-size air pumps at the vacuum required. A Nelson pump or a water aspirator has been found sufficient to handle the continuous air flow through 2 or 3 jets, while industrial-size vacuum

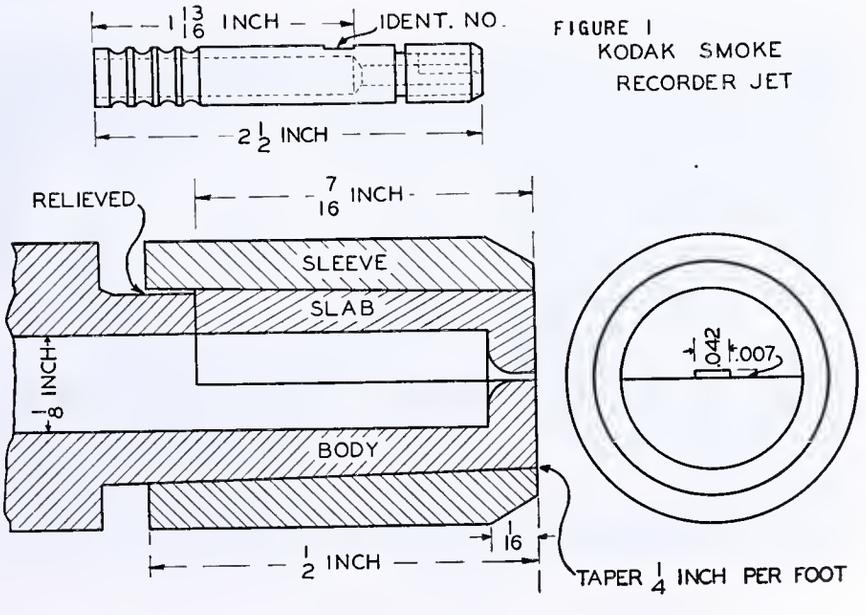


FIGURE 1
KODAK SMOKE
RECORDER JET

FIGURE 2. CONSTRUCTION OF JET

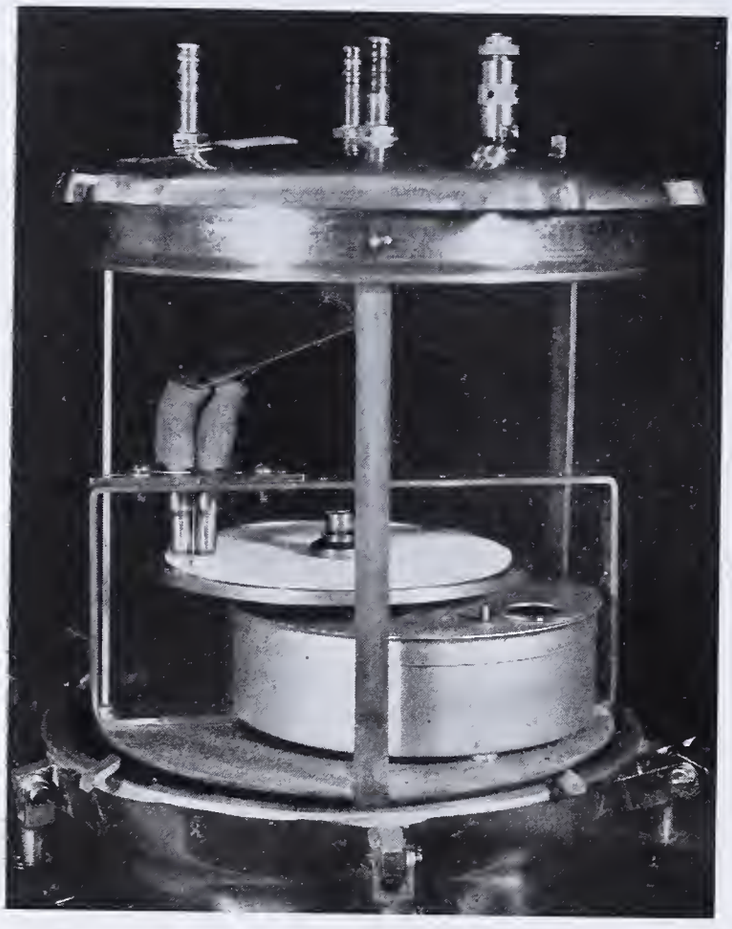


FIGURE 3. IMPINGING AND RECORDING MECHANISM

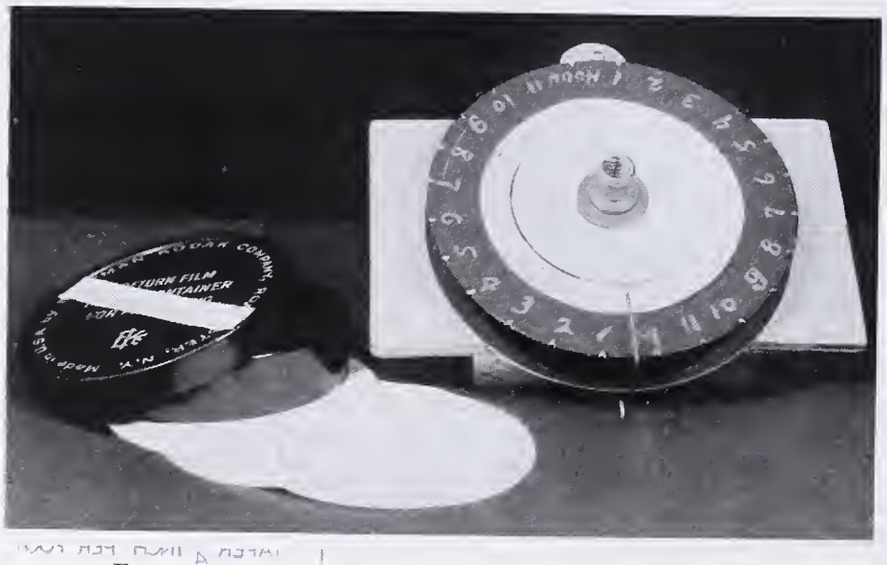


FIGURE 4. APPARATUS FOR VISUALLY MATCHING TRACES

pumps of high capacity operating at 0.53 atmosphere can accommodate many jets. A wider jet gives records which are easier to read but the vacuum pump capacity then required is difficult to meet. As a precaution against accidental plugging, an orifice flowmeter is used regularly to verify the amount of air passed by the jets. Occasional cleaning of the jets is necessary. In this laboratory a chart less than 15 cm. (6 inches) in diameter is usually employed. Where the air is unduly dirty a larger chart can be used and the diameter of the trace enlarged to diminish the densities. Use of an 8- rather than a 24-hour clock will have the same effect.

To prevent lint and other relatively large air-borne particles from plugging the jet, a bolting silk screen (120-mesh, 0.63-cm., 0.25-inch, diameter) is held in the body of the jet between two lengths of 0.63-cm. (0.25-inch) outside diameter brass tubing. In this laboratory it was found necessary to change the screens no oftener than once daily when using outdoor air. The dirt removed by the bolting silk screen is of sufficiently large size to be filtered out in practice by any reasonably efficient filter. Such large particles can be separated by sedimentation, a technique often used in reporting the results of surveys concerning atmospheric pollution (*1*, *8*).

It was found that a 9.5-liter (10-quart) aluminum pressure cooker met the requirements as a container for the impinging and recording mechanism. The clock and jet assembly are suspended from the cover, making the recorder easy to service by removal of the top. The air inlets and outlet are tapped through the cover as shown in Figure 3.

Evaluation of Records

Qualitative and semiquantitative results can be obtained in air-filter studies by matching traces with a standard gray scale by reflected light. This suffices for qualitative work. In Figure 4 is shown a rough apparatus for this purpose and a typical chart. Standard gray scales can be prepared by exposing and developing matte-surface photographic paper to various degrees of blackness and then determining reflected densities by means of a densitometer. These reflection densitometer readings were converted into reflection "dirt units", which are defined as the products of the densities and a constant. This constant is a factor which converts the density reading to a 0–100 scale for ease of mathematical manipulations. Its value depends upon the upper limit of density chosen for the investigation. Each laboratory must determine this constant; its absolute value is not a matter of great importance, however, since the purpose of the test is almost always measurement of the "smudging power" of dirt present in air.

Quantitative results can be obtained by the use of a modified Capstaff-Purdy densitometer (*4*) equipped with a slitlike aperture. A photoelectric transmission densitometer (Figure 5), which was developed in conjunction with an extensive survey of the efficiencies of different types of air filters, has made possible rapid and accurate reading of charts without the eye fatigue which accompanies the use of a comparator or a visual densitometer, such as the Capstaff-Purdy.

The optical system of the photoelectric densitometer is shown in Figure 6. In order to read the densities of the traces, the charts are placed between the box and the hinged head, so that the trace covers the 9-mm. slit. This slit is of such curvature that the reading represents an integration of a 1-hour record. The current produced in the phototube as a result of the light transmitted by the record is conducted to the circuit shown in Figure 7. The use of two photocells, one in each arm of the Wheatstone bridge, minimizes errors due to fluctuations in energy from the light source. The diaphragm covering the photocell contained in the box is necessary to equalize more nearly the amount of light falling on each tube.

Calibration of the instrument was accomplished by photographic step tablets with a wide range of densities. During calibration a piece of Whatman No. 50 filter paper was placed between the step tablet and the slit aperture to simulate

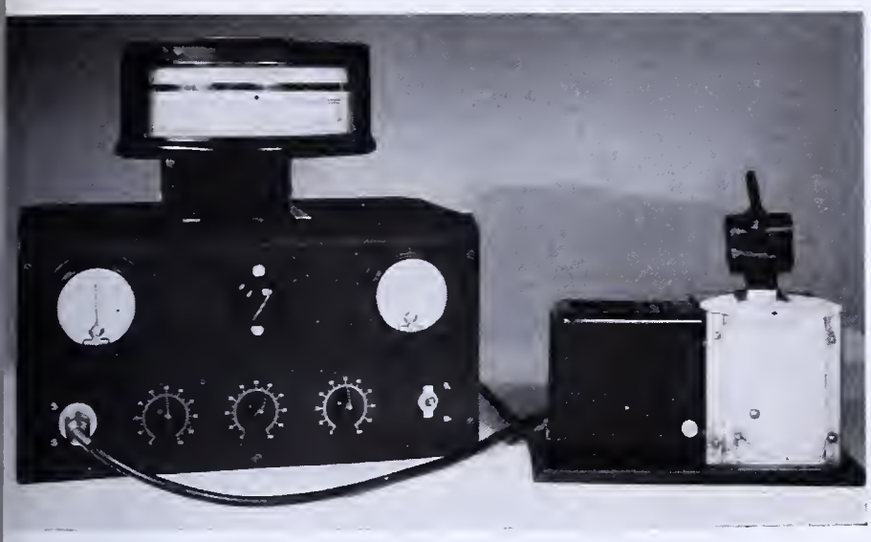


FIGURE 5. PHOTOELECTRIC TRANSMISSION DENSITOMETER

more nearly the actual conditions encountered while reading recorder charts. It has been found recently that matte Eastman acetate sheeting is more uniform in texture than paper and is more satisfactory than paper for recording light traces, where the zero correction is a matter of importance. These transmission densitometer readings, known as dirt units, when subtracted from 100 give the transmission value for the corresponding dirt trace.

It is customary in this laboratory to calculate air filter efficiencies by totaling separately the hourly dirt units for filtered and unfiltered air over a 24-hour period. The efficiency is calculated by the equation:

$$\text{Percentage efficiency of air filter} = 100 \times \frac{\text{daily total dirt units for unfiltered air} - \text{daily total dirt units for filtered air}}{\text{daily total dirt units for unfiltered air}}$$

Use of Kodak Smoke Recorder

From the previous discussion, it is obvious that the recorder is well suited to the study of air filtration and determination of the times of occurrence of heavy smoke loads; however, it has been found useful for other studies involving the purity of air. Spot tests have been used to determine by chemical means whether suspected contaminants, other than soot, were present. In this case simple circular jets of brass or glass can be used instead of the rectangular jets, which are more difficult to construct. When the particulate matter is of low concentration and when the time of occurrence is not important, a stationary paper can be used, and thus the air-borne material can be impinged upon a small area in order to concentrate the contaminant for chemical analysis. Transparent dusts are collected readily for microscopic examination on glass plates, cellulose acetate sheeting, or microscope slides set in special holders. This instrument is not designed for public health studies, since counts of siliceous dust are of value only when made with instruments previously used and for which there is an established body of experience.

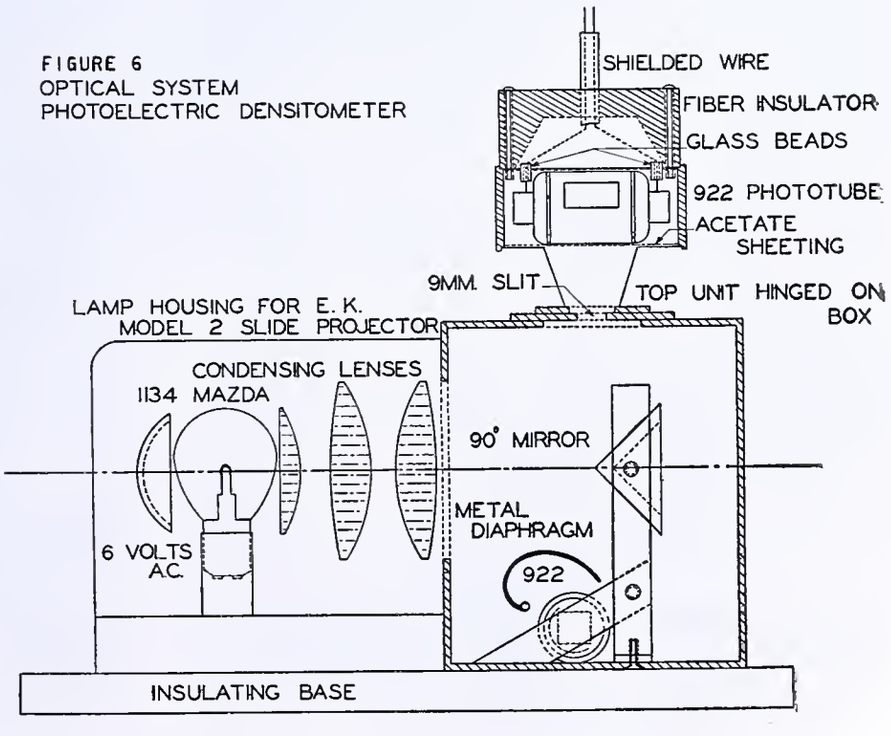
Advantages

The efficiency of the deposition process in the Kodak smoke recorder has been shown by several methods to be very high, and the simplicity of the apparatus permits 24-hour unsupervised operation. The instrument is easily portable and can be put into operation rapidly to take readings at widely separated locations. In this respect it surpasses the Dill–Bureau of Standards (*5*) method, which requires a laboratory setup and the almost constant attention of an operator during the test.

The cost of constructing the recorder is comparatively low and it is far cheaper and more compact than the equipment of Hazard (*7*), which is designed especially to preserve traces for microscopic examination. With reasonably careful handling the charts from the Kodak smoke recorder can be evaluated and preserved as permanent records. The Owens automatic air filter (*10*) is designed primarily for studies of atmospheric pollution. It operates by filtration through paper and is less sensitive, producing discontinuous records estimated to be $^1/_{35}$ as dark as those obtained by the recorder described herein. From 4 to 6 spots are produced per hour by the Owens apparatus. Drinker and Hatch (*6*) describe a number of techniques which apply more to industrial dusts than to smokes and smoke removal, as do Bloomfield and Dallavalle (*2*, *3*).

Since the Kodak technique measures the smokelike particles in air, or in effect the smudging power of the air-borne dirt, the filter efficiencies obtained will not agree with those determined by the American Society of Heating and Ventilating Engineers (*9*) and other methods. This, however, is not a serious matter—the A. S. H. V. E. method uses a synthetic mixture of lampblack and coal ash as a test material and is designed to measure efficiencies of viscous-type air filters under conditions where relatively coarse dirt must be removed, while the Kodak technique is designed to measure the efficiency of removing the finest smoke particles as well. The usefulness of this technique is demonstrated by the consistent data obtained in studying the efficiencies of air filters having ratings of about 90 per cent on the Kodak scale, and in measuring the effect of improvements made upon such filters. This is true since the record obtained from efficiently filtered city air by the use of most recorders is so faint that small differences cannot be perceived.

FIGURE 6
OPTICAL SYSTEM
PHOTOELECTRIC DENSITOMETER



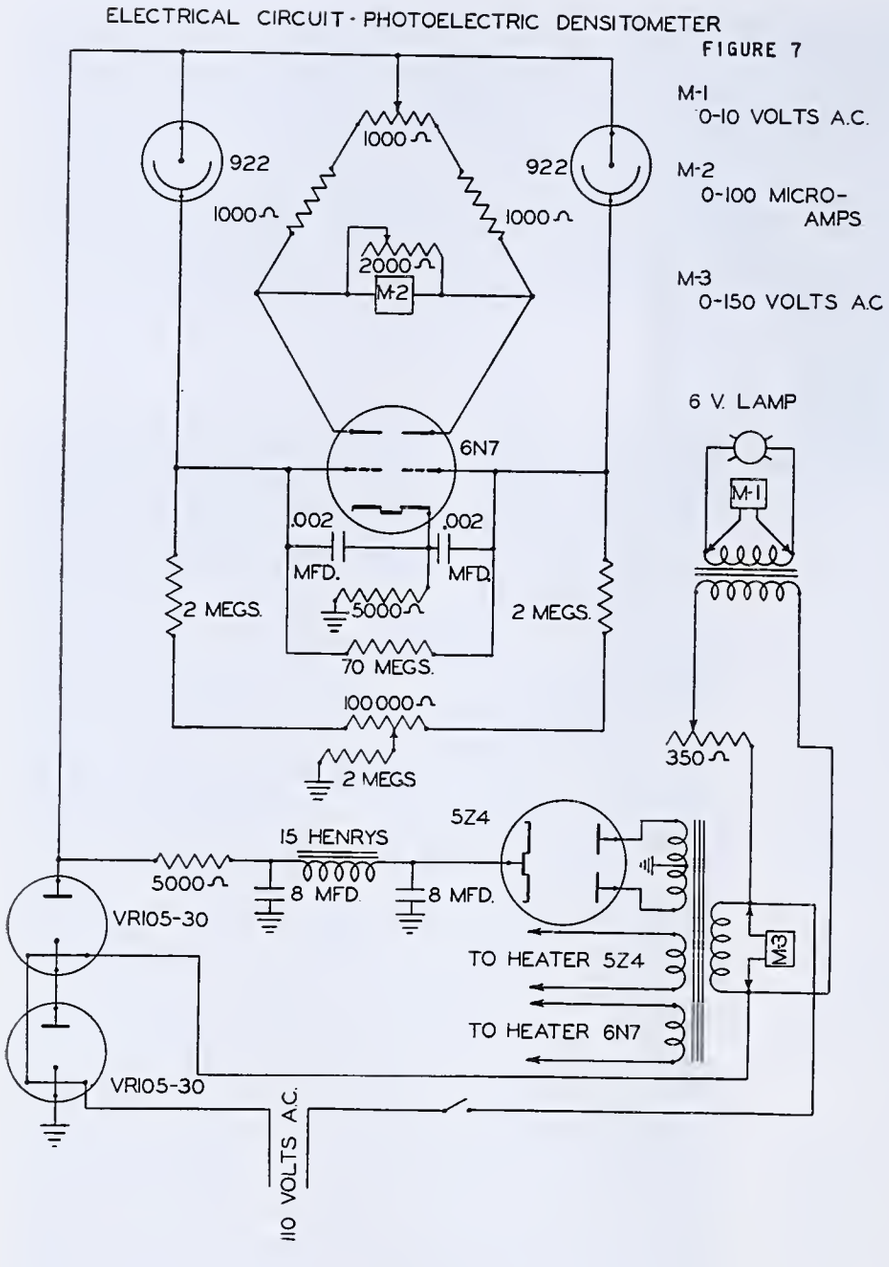


Table I is presented to describe the Kodak smoke scale of filter efficiencies, rather than to place various filters in any order of merit. For ranking purposes, one must also consider operating conditions. All filters mentioned were operated on outdoor air at the velocities recommended by manufacturers and over a range of practical pressure drops.

TABLE I. EFFICIENCY RATINGS

Filter	Dill–Bureau of Standards	A. S. H. V. E. Method	Kodak Method
	%	%	%
Viscous cell (wire, glass, etc.)	[a]	80–90	5–10
Spray chambers	[a]	80–90	5–10
Canton flannel bags	20–30	95–100	20–30
Deep beds of cotton (0.5 to 2 inches thick)	70–90	98–100	60–80
Electrostatic	90–100	100	90–100

[a] This method gives poor results below 20%.

Summary

Some modern industrial processes require very pure air, which is not easily measured with existing equipment; there are no commercially available instruments that give continuous readings.

The smoke-recording instrument described is easily portable and will give 24-hour records without supervision. It functions by impinging air from a jet at very high and uniform velocity upon a piece of paper driven by a clock in an evacuated vessel. At a pressure drop of 0.53 atmosphere the jet is self-metering, thus avoiding air-measurement difficulties.

The instrument is designed primarily for studying air filters of very high efficiency, but can also be used for chemical tests and for collecting specimens for microscopic examination.

A photoelectric densitometer is described. The impingement charts from the recorder can be read with this instrument and air-filter efficiencies determined by simple calculations.

The Kodak smoke scale is not absolute. Its character is shown by comparisons with the A. S. H. V. E. and Dill–Bureau of Standards methods for different filter types.

Acknowledgments

Thanks are due E. K. Carver, superintendent of the Department of Manufacturing Experiments, and C. K. Flint, manager of Kodak Park, for permission to publish this description of the Kodak smoke recorder and the technique of air-filter evaluation. A. F. Pundt and J. R. Peer are to be thanked for conscientious evaluation of many thousands of recorder charts. J. R. Peer and E. J. Klodinski constructed the photoelectric densitometer. The novel features of the Kodak smoke recorder have been covered by a patent application.

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A. C. S. Analytical Reagents

THE specifications for analytical reagent chemicals, recommended by the Committee on Analytical Reagents, AMERICAN CHEMICAL SOCIETY, from 1924 to 1941, have been reproduced in a planographed volume in the general style of the abstracts of papers presented at AMERICAN CHEMICAL SOCIETY meetings.

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Particle Size Determination by Sedimentation

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THERE are many occasions when the ordinary screen analysis of granular material does not permit sufficiently fine differentiation between particle sizes. In such circumstances, sedimentation methods offer a means of obtaining as high a degree of differentiation as is desired.

Several different experimental methods of measuring particle size by sedimentation have been used but probably the simplest is that used by Kelly and others (*2*, *5*), which consists essentially of measuring the change in pressure exerted by the suspension as the suspended material separates out. Generally, in order to measure small pressure changes, an inclined manometer attached to the settling tube is used.

Evaluation of Experimental Data

The relationship between the pressure changes and the weight of material separated out may lead to serious errors in the particle size determination if the physical dimensions of the apparatus are not considered. When the pressures are indicated by the positions of the meniscus in the inclined arm of the manometer, which is filled with the suspending medium, the relationship as given by Kraemer and Stamm (*4*) is:

$$W_P = \frac{ds\,(S + A \sin b)}{s - d}\,(l_0 - l_t) = k_1 \Delta l \qquad (1)$$

where W_P = weight of material that has settled out at time t
d = density of suspending medium
s = density of suspended solid
S = cross-sectional area of bore of inclined arm of manometer
A = cross-sectional area of settling tube
l_0 = meniscus reading at $t = 0$
l_t = meniscus reading at time t

When S is so small compared to A that it can be neglected, Equation 1 reduces to that given by Kelly (*2*), except that Kelly's equation gives the amount in suspension at any time.

$$W_I = \frac{dVs \sin b}{h\,(s - d)}\,(l_0 - l_t) = W_0 - W_P = k_2 \Delta l \qquad (2)$$

where W_I = weight of material in suspension at time t
V = volume of suspension above side tube
h = height of suspension above side tube
W_0 = total weight of solid initially in suspension of volume V

Physically, this omission of S means that the amount of liquid entering the settling tube from the inclined arm is neglected.

If it is desired to evaluate the sedimentation data by means of Equation 2, the dimensions of the apparatus have to be adjusted accordingly. This can be done by making area A very large compared to area S. But for ordinary values of S (diameter 1 to 5 mm.) this means that the apparatus will be unduly large. On the other hand, if S is made small compared to a convenient value for A, difficulties and erroneous results may be encountered because the suspending medium will move too slowly in the inclined arm, owing to capillary attraction. Therefore, the safest procedure is to use Equation 1 in evaluating the data. Values of k_1 and k_2, obtained with an inclined manometer type of apparatus at the Drexel Institute of Technology, were 0.531 and 0.417, respectively, when operating with aqueous suspensions of calcium carbonate and with such a difference it is imperative to use Equation 1.

Another relation for the weight of material separated out and the pressure changes is that given by Duncombe and Withrow (*1*):

$$\% W_P = \frac{l_0 - l_t}{l_0 - l_f} \times 100 \qquad (3)$$

where l_f = final meniscus reading.

Despite its simplicity, the difficulties involved in the determination of l_f limit the general usefulness of this relationship.

Operating Variables

An examination of Equation 1 shows that the factors which are constant for any one experiment and apparatus are the densities of liquid and solid and the areas, while l_0 and l_t are the only variables that have to be measured. The various W_P's are calculated from the l readings and the sum of these values for the total settling time should equal the weight of solid initially in the suspension.

The determination of l_0 may offer some difficulties. In the determination of the total meniscus change the relationship between the initial level of the suspension and the level of the meniscus exerts considerable influence at the start of the experiment. Although the relative position of these two levels is usually approximated prior to a settling experiment, it is almost impossible to have them in their exact positions in the actual determination. Therefore, the meniscus level is either somewhat above the actual level corresponding to the level of the suspension in the settling tube, or it is somewhat below the actual level. In the first case the meniscus level will drop at a rate faster than that corresponding to the settling out of particles and in the second case it will drop at a rate lower than the actual rate. In extreme instances of the second case the meniscus may actually be observed to stand still or even move upward for a short time. It is, therefore, necessary that the actual meniscus level be determined graphically by plotting meniscus readings *vs.* time and extrapolating to zero time. Generally, it will be found that meniscus readings below 1 minute are not reliable, and it may therefore be of advantage to take meniscus readings at intervals of 30 seconds or less in the period of about 1 to 5 minutes. This procedure should give sufficient points of the time *vs.* meniscus reading curve to permit accurate extrapolation to zero time.

The initial meniscus position can also be found from Equation 1 when W_P and k_1 can be determined accurately because $\Delta l = l_0 - l_t = \frac{W_P}{k_1}$. In this case it is best to measure Δl for complete settling because then W_P is the total weight of solid in suspension.

In using Equation 1 it is desirable to know W_0 in order to check the accuracy of the summation of the individual weights corresponding to the l readings. When Equation 2 is used, it is necessary to know W_0 accurately. If the suspension is uniform and the total volume of the settling tube and the volume of the tube above the side arm (all to the same height) are known, W_0 may be found from the weight of material put into the settling tube. In some cases, k_1 and Δl for complete settling may be found accurately enough to permit determining W_0 from Equation 1. Of course, an aliquot of the suspension could be analyzed, but again it would be necessary to assume a uniform suspension.

Viscosity of Suspending Medium

The viscosity of the suspending medium is primarily determined by the largest particle size which is to be measured. If the viscosity of the medium is too low, particles above a certain size will settle so rapidly that differentiation will not be possible and if it is necessary to obtain closer differentiation a medium of higher viscosity must be used. The viscosity which is required can be approximated from Stokes' law if an estimate of the largest particle size is available. According to Knapp (*3*), the minimum allowable viscosity is given by:

$$\mu_{min.} = \sqrt{0.37\, r^3_{max.}\, gd\,(s - d)} \qquad (4)$$

where $r_{max.}$ is the maximum particle radius, g is the gravitational constant, and the other terms are as previously defined.

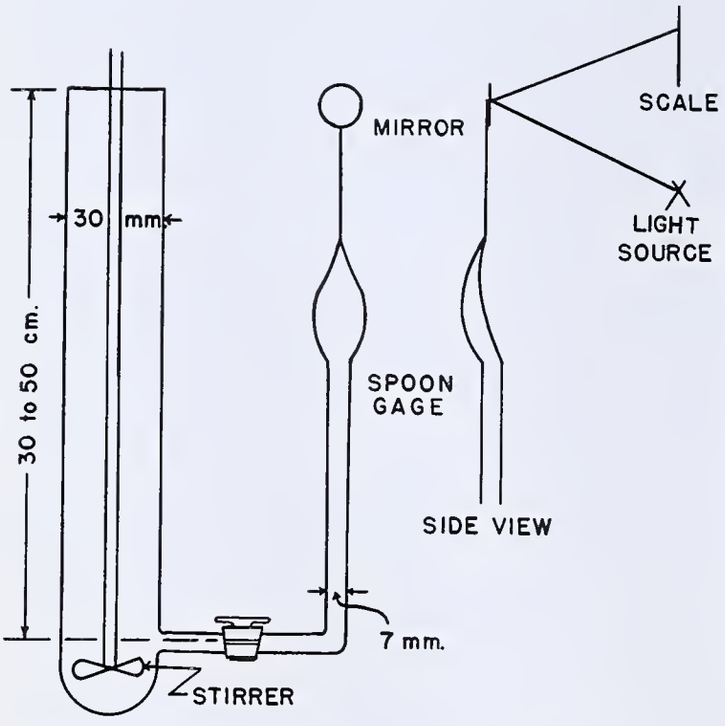

FIGURE 1. APPARATUS FOR PARTICLE SIZE DETERMINATION

Occasionally it was observed that the curve of weight settled *vs.* time showed a curvature which was slightly concave upward in the early part of the settling period. An analysis of a number of such curves indicated that the occurrence of this inverted section of the curves was found with solids of a wide particle size range which contained a considerable percentage of large particles. The slope of this part of a curve, of course, indicated that the reverse of sedimentation was taking place. Close observation of the settling process revealed that during the period of rapid settling—that is, in the part represented by the steep portion of the settling curve—the liquid which returned from the semicapillary tube entered the settling tube with sufficient velocity to set up an upward current. As a consequence some particles which had settled past the level of the side arm were lifted above this level and immediately exerted an increased pressure which resulted in a slowing up of the rate of meniscus fall. Translated into the weight *vs.* time curve this means a decrease in the slope dW/dt and when normal settling is resumed a discontinuity is introduced in the settling process. The remedy for such a condition is the use of a suspending medium of higher viscosity which will retard the rate of fall of the meniscus to a point where the liquid draining from the semicapillary tube will not set up eddy currents.

Smallest and Largest Particle Sizes

When, in a particular experiment, a suspending medium is used which has a viscosity high enough to permit accurate measurement of the fall of the majority of the particles, it will usually be found that for this medium the smallest particles settle too slowly and the largest too quickly. The size of the smallest particles can generally be found accurately enough by extrapolating the accumulation curve of weight settled out *vs.* time to complete settling and using this value of the time in the Stokes' equation. This curve becomes asymptotic to a line parallel to the time axis but the error introduced by extrapolating to 100 per cent settled out is small since the radii of the particles are small.

It is more difficult to arrive at the size of the largest particles. In the usual case they will generally settle so rapidly that accurate measurements cannot be made at less than one minute. As an approximation it is possible to extrapolate the curve to the origin with a straight line and in some cases this is accurate enough. Of course, a screen analysis will also indicate the upper limit of particle size. The other, more accurate, procedure is to use a medium of higher viscosity for these particles, so that the time of fall is increased sufficiently to permit accurate measurement.

Improved Apparatus

The discussion of the above factors shows that the methods which are based on a moving meniscus level involve experimental difficulties which may result in appreciable inaccuracies. Furthermore, with large particle sizes these methods are of little use because suspending media of high viscosity must be used to permit accurate measurement of the time of fall of the particles. On account of the high viscosity, the liquid moves so slowly in the capillary side arm that the measurements are practically worthless.

The apparatus shown in Figure 1 was developed in the laboratories of the Pure Oil Company. It permits the use of a suspending medium of high viscosity and with it particle sizes corresponding to 32-mesh (about 500 microns in diameter) have been measured employing an S. A. E. 40 oil as suspending medium.

The settling tube is large enough to permit the introduction of a motor-driven stirrer arranged to give an upward motion to the suspension. The spoon gage, made by elongating a thin-walled bulb and then flattening on one side, is thin enough so that a pressure difference of a few millimeters of mercury will impart a noticeable displacement to the pointer. The optical arrangement amplifies the pointer movement.

In use, the whole apparatus is filled with the suspending medium to a fixed point on the settling tube. After closing the stopcock, a weighed amount of the material under investigation is stirred in until the suspension is uniform. Upon cessation of stirring, the stopcock is opened and readings of the pointer position are taken at various times. The apparatus must be protected from thermal and mechanical changes.

The deflections of the pointer are converted to pressure changes by calibrating the gage. This can be done, as described by Knapp (*3*), by immersing cylinders of known volumes in the suspending liquid in the settling tube. The increases in height of the liquid are converted to pressure increases and these are plotted against the corresponding pointer displacements. The resulting curve is a calibration curve of the gage.

From the pressure changes the weight of material separated out is found by Equation 5.

$$\Delta P = \frac{W_P}{A}\left(1 - \frac{d}{s}\right) \qquad (5)$$

in which the symbols have the same meaning as before. Obviously, the percentages settled out can be obtained also

by means of Equation 3. After constructing the accumulation curve, the particle size distribution curve is obtained in the usual manner.

There are many advantages of this apparatus in actual use. Since a given height, h, of the liquid always corresponds to a definite position, l, of the pointer it is apparent that, knowing the bulk density of the material (determined with the liquid which is used as the suspending medium), the final position of the pointer, l_f, can be calculated. This corresponds to complete settling. Since there is no flow of liquid from the side arm, disturbances which can be caused in this way in the manometer type of apparatus are eliminated. Because of the amplification of the pointer displacement, closer differentiation of particle sizes is possible.

Experience has shown that this apparatus is more convenient in use than the usual type, and particle sizes of materials can be measured with it that are impossible to measure with the manometer type.

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Presented before the Division of Industrial and Engineering Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Recovery of Mercuric Iodide and Iodine from Nesslerized Solutions

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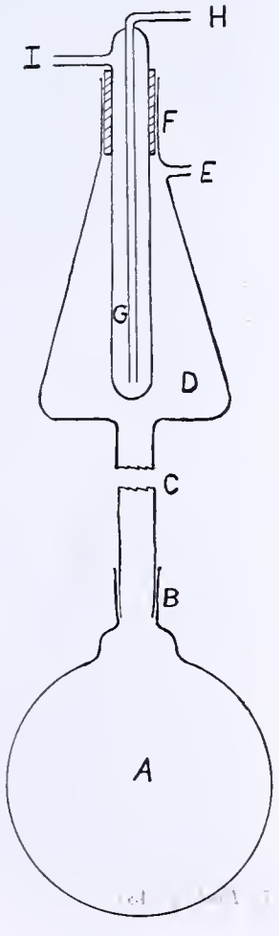
BECAUSE of the rising cost of mercury, it seemed advisable to investigate its recovery from the large quantities of nesslerized solutions prepared in the course of running hundreds of nonprotein nitrogen determinations according to the Koch-McMeekin method (*2*).

Pullman (*4*) recovered the mercury and iodine by neutralizing the nesslerized solution with sulfuric acid and adding mercuric nitrate equivalent to the amount of mercury already present. He stated that all the mercury precipitated as mercuric iodide. However, this method involved the use of a second mercury compound which required a rather large additional expenditure in order to recover the original mercuric iodide. Clifford (*1*) recovered mercuric iodide by neutralizing the nesslerized solution with sulfuric acid. Only a part of the mercuric iodide precipitated from the solution on standing.

In this investigation mercuric iodide and free iodine were precipitated by adding sulfuric acid and sodium dichromate to the nesslerized solution. The free iodine was then separated from the mercuric iodide by a distillation procedure. The method is simple and inexpensive, and results in the almost quantitative recovery of iodine and of mercury as mercuric iodide.

Apparatus

The apparatus consists of a 3-liter round-bottomed short-necked flask, *A*, with standard-taper ground-glass joint 25/40, *B*, connected by a 50-cm. (20-inch) length of 20-mm. (outside diameter) Pyrex tubing, *C*, to an opening in the bottom of a 1-liter Erlenmeyer flask, *D*, which has a side arm, *E*, attached near the top. Through the mouth of the Erlenmeyer flask, *F*, there extends a cooling finger, *G*, which contains a running water inlet, *H*, and outlet, *I*. *G* is fitted into *F* by means of a rubber washer which must be renewed occasionally—a glass connection would be preferable.



Procedure

To 10 liters of the nesslerized solution contained in an earthenware crock are added, in the order given, 150 cc. of concentrated sulfuric acid and 75 cc. of 1.3 molar sodium dichromate solution. Upon addition of the sulfuric acid the mixture first becomes milky, then develops a pink color. Upon addition of the sodium dichromate solution, the color darkens considerably and a small amount of iodine vapor becomes noticeable. The mixture is covered and allowed to stand at room temperature in the hood for 12 hours, during which time the mercuric iodide and iodine are completely precipitated. The supernatant liquid is decanted and the residual mass transferred to flask *A*. (In case the nesslerized solution has been prepared in the course of urea determinations in which a direct nesslerization procedure has been used, it is advisable to free the precipitate from the supernatant liquid by suction filtration and to wash the residue with water prior to its transfer to the round-bottomed flask. This procedure removes the organic material which causes foaming during the heating process.) Enough water is introduced in transferring the precipitate to fill the flask one-fourth to one-half full. (The water facilitates the rapid removal of free iodine from the mixture of mercuric iodide and iodine during the subsequent procedure.)

The apparatus is assembled as shown. The mixture is boiled gently until *A* is free of iodine vapors. The apparatus is then disconnected and the free iodine, which has condensed on the cold finger, is scraped or washed into a beaker, collected in a Büchner funnel, and allowed to dry in the air. The mercuric iodide remaining in *A* is separated by suction filtration and washed well with distilled water and then with three 25-cc. portions of 95 per cent ethyl alcohol. It is heated in an oven at 105° C. for 30 minutes in order to remove any moisture or free iodine that may still be present. The mercuric iodide is then ready for use in the preparation of Nessler-Folin reagent.

Results

From 54 batches of nesslerized solution, 10 liters each, an average of 45 grams of iodine and 84 grams of mercuric iodide was recovered. The calculated amounts present in the 10 liters were 48.8 grams of iodine and 87.7 grams of

mercuric iodide. Percentage yield: mercuric iodide, 95.7; iodine, 92.2.

PREPARATION OF NESSLER-FOLIN REAGENT. The Nessler-Folin reagent is prepared according to the method of Koch (*3*) except that 40.3 grams of the recovered mercuric iodide (instead of 30 grams of mercury and 22.5 grams of iodine) are added to 30 grams of potassium iodide in 30 cc. of water. The resulting solution is filtered, adjusted with a solution of iodine in potassium iodide, and diluted to 200 cc. This stock solution is then added to 975 cc. of 2.5 molar sodium hydroxide to give the Nessler-Folin reagent. No difference has been observed in the behavior of the solutions prepared by the two methods.

The recovered iodine has been used successfully in the preparation of Nessler-Folin reagent according to the method of Koch (*3*).

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Determination of Iodate Ion in the Presence of Cupric Ion

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FOR a number of routine determinations a method for estimating iodate ion in the presence of copper salts was required. Of the methods suggested for the estimation of iodates of alkali metals or iodic acid alone, the most important are: reducing iodates with oxalic acid and back-titrating the excess of oxalic acid (*1*), reducing iodates with titanous chloride (*3*), and allowing the iodate and potassium iodide to react in the presence of a mineral acid and titrating the liberated iodine against standard sodium thiosulfate solution (*5*). The last reaction is sufficiently rapid and accurate for all purposes. The velocity of the reaction

$$6H^+ + IO_3^- + 5I^- \longrightarrow 3I_2 + 3H_2O$$

in acetic acid solution has been shown to be proportional to the square of the concentration of hydrogen and iodide ions and directly proportional to the concentration of iodate ion (*2*). This reaction has also been utilized for estimation of iodate ion in the presence of bromate and chlorate ions. If, however, estimation of iodate ion be attempted in the presence of copper ions, a complication is likely to arise on account of the liberation of iodine through the simultaneous reaction of cupric ions with potassium iodide according to the equation

$$2Cu^{++} + 2I^- \longrightarrow 2Cu + I_2$$

Kolthoff and Cremer (*4*, *7*) showed that trivalent arsenic can be estimated volumetrically in the presence of copper ions by adding sodium pyrophosphate to a neutral solution of the mixture, when copper ions form a blue complex and no longer react with potassium iodide. It was found, however, that the complex of copper does not decompose in acidic solution up to pH 5.0, but decomposes at greater acidity to react with potassium iodide. The authors made use of this fact for the estimation of iodate ion in the presence of copper salts.

Mixtures containing known amounts of potassium iodate and copper sulfate were prepared. An excess of sodium pyrophosphate (free from reducing agent), acetic acid, and potassium iodide solution were added, in this order, to each of the solutions to be titrated. The iodine was liberated slowly and was titrated against standard sodium thiosulfate solution, using starch as indicator. After iodine had ceased to separate, the solution was set aside and kept for 24 hours in the dark. Under these conditions no more iodine was evolved, showing that the blue copper complex did not decompose to react with iodide.

In Table I are given typical results obtained in the course of this investigation. In the fifth column are given the exact quantities of various solutions that were found to give the most consistent results.

The results given in the last column show that the amount of iodate added to the solution in terms of its equivalent iodine can be estimated with accuracy even in the presence of very large amounts of copper salts. The only difficulty encountered in the present case was the extreme slowness with which the reaction proceeded, each titration taking several minutes, sometimes even half an hour, for completion. A number of catalysts, including ammonium molybdate (*6*), which has been mentioned by Kolthoff as suitable for catalyzing the liberation of bromine from a mixture of bromide and bromate, were tried without success.

The kinetics of the reaction are being studied and will be published shortly.

TABLE I. TYPICAL RESULTS

A. Sodium pyrophosphate solution, 10%. B. Acetic acid solution, 10%. C. Potassium iodide solution, 10%.

No.	Volume of KIO_3	Concentration N	Volume of 0.2 N $CuSO_4$	Conditions	Volume of 0.1 N $Na_2S_2O_3$ Theoretical	Found
	Cc.		*Cc.*	*Cc.*	*Cc.*	*Cc.*
1	25	0.1	..	5 B 10 C	25.0	25.0
2	25	0.1	..	75 A 12 B 10 C	25.0	25.0
3	25	0.1	10	10 C	25.0	25.0
4	25	0.1	25	10 C	25.0	25.0
5	50	0.1	50	150 A 25 B 20 C	50.0	50.05
					Volume of 0.01 N $Na_2S_2O_3$	
6	5	0.01	25	Same as in 4	5.0	5.0
7	10	0.01	25	Same as in 4	10.0	10.0
8	25	0.01	25	Same as in 4	25.0	25.05

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A Polarographic Study of Aliphatic Nitro Compounds

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A POLAROGRAPHIC study was made of six aliphatic nitro compounds with the expectation of developing a method for the quantitative determination of these compounds. The investigation also included a determination of the half-wave potentials. The current due to the reduction of the compounds in acid solution was found to be proportional to their concentration, but in neutral solution there was a slight but reproducible deviation from this linear relationship. The compounds were reduced at a lower potential in acid than in neutral solution. The ease of reduction increases slightly with increase of molecular weight, and is greater for primary than for secondary compounds.

Apparatus and Materials

The polarographic method of analysis has been described by different investigators (*3*, *4*). The current-potential curves may be obtained with an automatic instrument which makes a photographic record, or by changing the voltage manually in certain steps and reading the deflection of a galvanometer at each voltage. The latter method is less convenient but is also less expensive and generally more accurate. In this investigation the manual method was used.

The apparatus was assembled from available parts. A Kohlrausch slide-wire bridge was used to vary the voltage across the cell. The voltage was measured with a Weston, model 45, voltmeter with a 3-volt range, which could be read accurately to 0.01 volt and approximately to 0.002 volt. A Leeds & Northrup galvanometer, HS type, was available with a coil with a 7-second period. Calibrated shunts were used in order that one fiftieth of the current should flow through the galvanometer—i. e., 0.13 microampere through the cell containing dilute solutions would cause a deflection of 1 mm. on the scale. With more concentrated solutions a shunt was used to give a sensitivity of one five-hundredth. A straight scale was used at a distance of about 2 meters from the galvanometer. Scale deflections were proportional to the current, since the maximum angular deflection was about 5 degrees. Current of the desired voltage was obtained from a lead storage battery.

The capillary cathode was prepared by drawing out a piece of thermometer tubing to an external diameter of about 1 mm., and was sealed to a piece of tubing with a reservoir at the top for the mercury. The end of the capillary was cut back to give a drop of mercury about every 5 seconds with a head of 25 cm. of mercury. Since the magnitude of the diffusion current varied with the drop time, the pressure upon the mercury was adjusted at the start of each series of experiments in order to obtain comparable results by using a 0.005 *M* solution of 1-nitropropane in 0.05 *M* sulfuric acid to give the same deflection each time at an applied voltage of 1.60 across the cell. Pressure was applied by using an aspirator bulb attached to a 1-liter flask and was measured with a mercury-filled manometer (*5*). The cell was a 50-ml. conical flask with a side arm sealed in. For some of the experiments dissolved air was removed from the solutions with nitrogen which had bubbled through a solution of chromous sulfate to remove traces of oxygen (*6*).

Five of the nitro compounds were available from members of the division of organic chemistry and had been purified by rectification in a modified Podbielniak column. The 1-nitrobutane was a good commercial grade. It was dried with Drierite and had a boiling point range of 152–154° C. In Table I are listed the boiling point ranges of the compounds which were used.

Experimental and Results

Solutions of each of the aliphatic nitro compounds were electrolyzed in 0.05 *M* sulfuric acid or sodium sulfate as indifferent electrolytes. Sodium sulfate solutions were used at the beginning of the investigation, but it soon became evident that a change of the compounds to the aci-nitro form was probably responsible for the lack of a linear proportionality between the concentration of the nitro compound and the wave height. The investigation was therefore continued with sulfuric acid solutions, in which case the wave height was found to be proportional to the concentration within a few per cent error. The stock solutions were 0.05 and 0.005 *M* solutions of the nitro compound in either 0.05 *M* sulfuric or sodium sulfate. The concentrations were varied from 0.0005 to 0.0167 *M* by mixing at the time of the experiment measured amounts of the stock solution with 10 ml. of the indifferent electrolyte.

The influence of pressure on the drop time and galvanometer deflection was investigated with a solution of 0.0046 *M* 1-nitropropane in 0.05 *M* sulfuric acid, with 1.60 volts across the cell.

The results tabulated in Table II indicate that it is important to have a reproducible pressure to obtain precision in the results, and this was accomplished as mentioned above. An analysis error of 1 per cent can be caused by a 1-mm. variation of pressure on the mercury of the capillary electrode.

The influence of temperature was determined by electrolyzing a 0.0167 *M* 2-nitropropane solution in 0.05 *M* sulfuric acid. The results tabulated in Table III indicate that a 2° variation in temperature will change the diffusion current by 3 per cent. All results reported in this paper were obtained with the cell immersed in a water bath at 25° ± 0.5° C.

The average of the oscillations caused by the growth and dropping of the mercury drops has generally been taken as the current when polarograms are obtained with a recording polarograph. In the manual method twice as many readings would be required. The use of the lower values of the oscillations was tried, and in plotting the wave height against the concentration of the compound, straight-line graphs were obtained which differed only in magnitude of deflection from those which were obtained when the average deflection was used. Since the minima of the oscillations were more reproducible, they were used to calculate the diffusion current from the galvanometer deflections.

In some preliminary experiments, current-potential curves were obtained for the electrolysis of 0.05 *M* sulfuric acid through which nitrogen had been bubbled to remove air. In those cases when the air had not been removed from the solution oxygen maxima were not observed, for which the authors cannot offer an explanation. A small but definite

Table I. Boiling Point Ranges

	° C.		° C.
Nitromethane	100.5	2-Nitropropane	117–118
Nitroethane	115	1-Nitrobutane	152–154
1-Nitropropane	130.8–131.6	2-Nitrobutane	139

Table II. Influence of Pressure on Drop Time

Pressure	Drop Time	Current	Change in Current for 1 Mm.
Mm. Hg	*Seconds*	*Microamperes*	%
240	6.5	3.45	...
250	5.6	3.79	1.0
260	5.0	4.09	0.93
270	4.5	4.35	0.90

Table III. Influence of Temperature on Current

Temperature	Current	Change per Degree
° C.	*Microamperes*	%
15	14.5	−1.4
20	15.7	−1.4
25	16.9	...
30	18.1	+1.4

Table IV. Diffusion Current in 0.05 *M* Sulfuric Acid

	Molar Concentration of Nitro Compound							
	0.000455	0.000833	0.00115	0.00167	0.00455	0.00833	0.0115	0.0167
	Microamperes							
Nitromethane	0.49	0.89	1.11	1.74	5.7	10.6	14.4	21.1
	0.51	0.89	1.25	1.79	5.4	10.3	14.2	20.7
Nitroethane	0.36	0.66	0.89	1.31	3.9	7.4	10.2	15.3
	0.40	0.67	0.89	1.29	4.2	7.6	10.5	15.0
1-Nitropropane	0.33	0.65	0.89	1.28	3.6	6.9	9.6	14.3
	0.35	0.64	0.88	1.29	3.8	6.9	9.7	14.2
1-Nitrobutane	0.34	0.65	0.93	1.34	..	..	..	..
	0.33	0.67	0.91	1.35	..	..	..	..
2-Nitropropane	0.39	0.70	0.98	1.39	4.4	8.1	11.3	16.4
	0.39	0.72	0.95	1.38	4.3	8.0	11.3	16.4
2-Nitrobutane	0.40	0.74	1.01	1.44	4.5	8.2	11.6	16.7
	0.40	0.74	0.99	1.48	4.5	7.8	11.4	16.9

Table V. Diffusion Current in 0.05 *M* Sodium Sulfate

	Molar Concentration of Nitro Compound				
	0.00455	0.00833	0.0115	0.0143	0.0167
	Microamperes				
Nitromethane	5.2	8.7	11.6	13.2	14.9
	5.2	8.4	11.1	13.3	15.0
Nitroethane	5.8	9.1	13.0	16.0	18.6
	5.2	9.5	12.7	15.4	17.5
1-Nitropropane	4.9	8.7	11.8	15.0	16.9
	4.7	8.5	11.9	14.5	16.8
2-Nitropropane	4.4	7.8	10.7	13.2	15.6
	4.4	7.9	10.8	13.3	15.5
2-Nitrobutane	4.3	7.6	10.3	12.7	14.7
	4.2	8.1	10.9	12.7	14.7

polarization current of increasing magnitude was obtained at increasing voltages. Hence, in most of the later experiments air was not removed from the solution but the curve was established for the electrolyte, then a desired amount of the compound was added, and the curve was again determined. The difference between the two curves was ascribed to the effect of the compound and gave the desired wave height. Deflections thus calculated were constant until a potential of 0.9 volt was reached, and attained another almost constant value at 1.4 volts. In sodium sulfate solutions the deflections were observed at 0.8 and 1.6 volts.

Two representative results from numerous data are tabulated in Tables IV and V for each concentration. These were obtained with a capillary electrode delivering 0.00162 gram of mercury per drop and with a drop time of about 5 seconds. The results show that the wave heights were proportional to the concentration of the nitro compounds in all the cases when sulfuric acid was used as the indifferent electrolyte. Straight-line relationships were not obtained when the compounds were dissolved in sodium sulfate solutions. Nitromethane showed the largest deviation, while 1-nitrobutane showed the least. The partial conversion of the nitro compound to an aci-nitro form in neutral solution and its complete conversion in an alkaline solution can explain the trend of the results. The half-wave potentials show that the nitro compounds were reduced more easily in acid than in neutral solution; in fact, they could not be reduced in 0.1 *N* sodium hydroxide. With tetramethylammonium hydroxide as the indifferent electrolyte a lower cathode potential could be reached, but even in this case no reduction was apparent. The formula of the aci-nitro ion which cannot be reduced is very likely $RCH{=}N\langle^{O-}_{O}$ according to Hantzch and others (*1*, *2*). In acid solutions the nitro compound is probably reduced to the oxime and not all the way to the amine, in view of the fact that only one wave height was observed in the voltage range which it was possible to use with sulfuric acid as supporting electrolyte. Further support to this idea is given by the fact that nitromethane is reduced to methyl hydroxylamine in acid solutions with zinc dust and can only be reduced further to methylamine with hydroiodic acid.

The half-wave or apparent reduction potential for a particular compound is defined as the potential of the cathode which will cause half the rise in current due to the substance in the solution and is usually considered to be independent of changes in the concentration of the compound as long as the hydrogen-ion concentration remains constant.

The half-wave potentials were measured with a Leeds & Northrup Type K potentiometer, using a mercury–mercurous sulfate electrode, 0.05 *M* in sodium sulfate, as reference electrode. Its potential was determined by comparing it with a normal calomel electrode through a salt bridge. Since oxidation-reduction potentials are often given with respect to other electrodes, primarily the normal calomel and hydrogen electrodes, the potentials listed in the last two columns of Table VI are with respect to these two electrodes. The results are tabulated for the dilute solutions. For the concentrated solutions the potential is approximately 0.01 volt more negative in the case of the sodium sulfate solutions and 0.04 volt in the case of the sulfuric acid solutions—i. e., the value 1.066 for 0.00046 *M* nitromethane became 1.106 for 0.0167 *M* solution.

The results show that there was not enough difference between the values of the half-wave potentials of the nitro compounds to determine them separately in the presence of each other.

Table VI. Half-Wave Potentials

	Applied Potential at Half-Wave	Cathode Potential Against reference electrode	Against *N* calomel electrode	Against hydrogen electrode
	In 0.05 *M* sulfuric acid			
Nitromethane	1.066	1.137	0.721	0.440
Nitroethane	1.048	1.115	0.699	0.418
1-Nitropropane	1.008	1.069	0.653	0.372
1-Nitrobutane	0.943	1.017	0.601	0.320
2-Nitropropane	1.052	1.129	0.713	0.432
2-Nitrobutane	1.022	1.097	0.681	0.400
	In 0.05 *M* sodium sulfate			
Nitromethane	1.23	1.35	0.93	0.65
Nitroethane	1.24	1.36	0.94	0.66
1-Nitropropane	1.23	1.35	0.93	0.65
1-Nitrobutane	1.21	1.30	0.88	0.60
2-Nitropropane	1.29	1.40	0.98	0.70
2-Nitrobutane	1.30	1.39	0.97	0.69

Summary

The reduction of six aliphatic nitro compounds at a dropping mercury cathode was studied with a manually operated polarograph in the concentration range 0.0005 to 0.017 molar. The deflections of the galvanometer were proportional to the concentration of the compound within a few per cent error. A 0.05 *M* sulfuric acid solution was used as the indifferent electrolyte. In neutral 0.05 *M* sodium sulfate solution a partial conversion to an aci-nitro form was probably the cause of the deviation from a linear relationship. The half-wave potentials were also determined, but it was not possible to determine the compounds separately in the presence of each other.

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Tracer Isotopes in Analytical Chemistry

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IN THE past, applications of physics and physical chemistry to analytical chemistry have afforded valuable new means for investigating and solving many problems of analysis. The use of isotopic atoms as tracers is the latest development to be added to an already impressive list, which includes many physical methods involving various optical, electrical, and thermal measurements. These tracer atoms are in general identical with the ordinary atoms of the elements except that they are "labeled" or "tagged" by virtue of possessing either a different atomic weight (or mass number) or the ability to undergo a spontaneous transformation to a different atom. This latter change, radioactivity, is accompanied by the emission of particles or radiation which can be readily detected or measured. Investigations employing tracer atoms thus fall naturally into two classes, those involving stable isotopes, which depend for analysis on a difference in mass, and those using unstable isotopes, the determination of which depends on the measurement of radioactivity.

Radioactive Tracers

Previous to 1934 investigations of analytical processes by radioactive means were confined to cases involving the naturally occurring radioactive isotopes (*23*). With the discovery of artificial radioactivity by Curie and Joliot (*6*) this new property of the common elements became available for studies of the various phases of analytical chemistry. At the present time radioactive isotopes of 87 elements have been artificially produced, making application to their analytical chemistry possible.

Preparation and Separation of Radioactive Elements

Some 330 artificial radioactive isotopes have been prepared by six kinds of bombardment of the naturally occurring atoms. These involve the bombardment of the target material by a stream of (1) neutrons (n), (2) protons (p), (3) deuterons (d), (4) alpha particles or He^{++} (α), (5) electrons, and (6) gamma rays produced in a suitable manner. The last two are unimportant for the production of useful quantities of the radioelements and will not be considered. With each of the first four kinds of projectiles, four or five different types of nuclear reaction are possible. Only one or two types of reaction for each kind of bombarding particle will be indicated [for a more complete discussion of this subject consult (*37*, *53*)].

Bombarding the same natural isotope of a given element with neutrons frequently results in more than one nuclear reaction, as, for example,

$$_{53}I^{127} + {}_0n' \xrightarrow{\text{slow}} {}_{53}I^{128} + \gamma$$
$$_{53}I^{127} + {}_0n' \xrightarrow{\text{fast}} {}_{52}Te^{127} + {}_1H^1$$
$$_{53}I^{127} + {}_0n' \xrightarrow{\text{very fast}} {}_{53}I^{126} + 2{}_0n^1$$

(The subscript indicates the atomic number and the superscript the mass number of the isotope.) Here we see that I^{128}, Te^{127}, or I^{126}, all of which are radioactive, is produced, depending on whether slow, fast, or very fast neutrons are used. These transformations today are more commonly represented as (n,γ), (n,p), and $(n,2n)$ reactions, indicating the projectile and the emitted particle, respectively, rather than by the more cumbersome equations above. Other neutron reactions that have been observed include (n,α) and (n,n) types. An example of the last one is In^{115} (n,n) In^{115*} where the asterisk indicates a radioactive isomer of the stable In^{115}.

Proton reactions are usually of the (p,n) type, though (p,γ), (p,α), and (p,p) are also known. The preparation of radiobromine is an excellent example of the (p,n) reaction:

$$_{34}Se^{80} + {}_1H^1 \longrightarrow {}_{35}Br^{80} + {}_0n^1$$

In the case of deuterons (d,p) and (d,n) reactions are most commonly obtained, with (d,α) and $(d,2n)$ less frequent. Typical of the first two are:

$$_{15}P^{31} + {}_1H^2 \longrightarrow {}_{15}P^{32} + {}_1H^1$$
$$_{16}S^{32} + {}_1H^2 \longrightarrow {}_{17}Cl^{33} + {}_0n^1$$

Five alpha-particle reactions have been observed: (α,n), (α,p), (α,d), $(\alpha,2n)$, and (α,α), the first of which is the most common. While the above bombardment of S^{32} with deuterons yields radio-Cl^{33}, bombardment with He^{++} gives radio-Cl^{34}:

$$_{16}S^{32} + {}_2He^4 \longrightarrow {}_{17}Cl^{34} + {}_1H^2$$

The half-lives of Cl^{33} and Cl^{34} are 2.8 seconds and 33 minutes, respectively. Thus, a given element may frequently be made radioactive in more than one way.

The best source of beams of protons, deuterons, and alpha particles for the above reactions is the "cyclotron" of Lawrence and Livingston (*33*, *34*). These same three particles may also be accelerated to high energies in the electrostatic generator (*22*). Neutrons are obtained by bombardment of beryllium with alpha particles from naturally occurring radioactive substances, such as radium, radon, and polonium, or as a by-product of a bombardment in the cyclotron or the electrostatic generator. Usually the projectile in the cyclotron is the deuteron and the target is beryllium, deuterium, or boron. The energy of the emitted neutrons depends on the nature of the target material and the energy of the deuterons.

Although the number of artificial radioelements known is large, the choice of a particular radioelement to use in an investigation is subject to a number of restrictions. Since these radioelements decay in accordance with the well-known exponential or first-order law, the time of half-life must be sufficiently long so that a conveniently measurable amount of radioactivity remains after completion of an experiment. Sometimes an element with a somewhat too short half-life (such as C^{11}, half-life = 21 minutes) may be used (*50*), provided a very high initial intensity can be obtained or a very sensitive instrument is available for quantitatively determining the radioactivity. Having tentatively selected an apparently suitable isotope, the means of preparation at one's disposal must be considered. Even though the various projectiles needed can be readily had, there still remains the question of the relative abundance of the natural isotope which must be bombarded to prepare the desired element. Thus, C^{14} is very readily prepared from C^{13} by the (d,p) reaction but C^{13} is present in ordinary carbon to the extent of only 1.1 per cent. The various restrictions indicated above seriously limit the number of radioactive isotopes actually remaining for use in chemical investigations, but the number available is still large.

Except when elements are transformed into radioactive isotopes of the same element, the elements produced in the transmutation process must be removed from the parent substance. In separations involving the radioactive elements, ordinary chemical and physical methods are generally applicable. No new chemical properties are conferred on an atom by virtue of its radioactivity, and the mass difference between stable and unstable isotopes is small enough to preclude appreciable variations in reactivity due to isotope effects.

Relatively few radioactive atoms are produced in the transmutation process. At such extremely low concentrations (of the order of 10^{-10} to 10^{-15} mole) many chemical procedures become uncertain. Some metallic sulfides, for example, have molar solubilities of the order of 10^{-12}. This corresponds to about 10^{11} atoms needed for precipitation, as contrasted with perhaps 10^{7} atoms produced in the transmutation process. Accordingly, the radioactive atoms could not be isolated by precipitation as the sulfide. Likewise, at such small concentrations, adsorption effects are more pronounced than at higher concentrations, as seen from the shape of the adsorption isotherm. Loss of active material could thus occur through adsorption on other precipitates formed during the separation process.

To ensure the efficient separation of the active isotope, a small amount of the corresponding inactive isotope is added after activation, and before making the separation. The active atoms are "carried" through the reactions necessary for their separation with a minimum loss. (Obviously, the active atoms cannot later be separated from the bulk of the inactive atoms added as carrier.) Occasionally, an active form can be isolated without using a carrier. In this case, the so-called specific activity (active atoms/total number of isotopic atoms) is at a maximum. Such separations frequently require no special methods other than those commonly used.

Chemical separation of active isotopes can be made with or without the use of a carrier.

In the preparation of radiosulfur, Voge (*64*) bombarded carbon tetrachloride with fast neutrons, obtaining radiosulfur in the form of elementary sulfur and various volatile, hydrolyzable compounds. The mixture was oxidized with alkaline hypobromite, a trace of sodium sulfate added as carrier, and barium sulfate precipitated from the acidified solution. Radiosulfur may also be prepared by bombarding sulfur with deuterons; no carrier sulfur is needed, as there is ample inactive sulfur left unaffected by the bombardment. Oxidation and precipitation as barium sulfate leave the active sulfur in a form readily converted into radioactive hydrogen sulfide.

Numerous methods are available for separating the radioactive halogens. The early method of Szilard and Chalmers (*61*) has recently been studied in detail by Lu and Sugden (*38*). The Szilard and Chalmers method consisted in irradiating liquid ethyl iodide containing a trace of free iodine with neutrons. A reducing agent was then added, and the iodine was converted to iodide and precipitated with silver nitrate. The precipitated silver iodide contained the bulk of the activity. In studying the method quantitatively, Lu and Sugden found that if free halogen was added to ethylene dibromide or butyl iodide before irradiation, the yield of extractable active isotope was better than when the free halogen is omitted. However, if phenyl bromide or phenyl iodide was irradiated, free halogen was without effect in increasing the yield. The halogen was extracted with aqueous solutions of acids, with sodium hydroxide or thiosulfate, or by metals (finely divided copper, silver, aluminum, and zinc). The addition of 4 per cent aniline before irradiation increased the percentage of radioactive material extractable by acids. Phenol likewise produced an increased, though smaller, extractability from water solutions. Presumably these compounds react preferentially with the radioactive bromine atoms liberated by gamma-ray recoil, enabling the active atoms to be extracted in highly concentrated form.

A method of interest in the preparation of radioactive hydrogen bromide, which depends on the complete and rapid exchange of bromine atoms with gaseous hydrogen bromide, has been given by Liberatore and Wiig (*35*). Radiobromine is produced by proton bombardment of selenium. When gaseous hydrogen bromide is heated with the powdered selenium, the radioactive bromine atoms exchange with the bromine atoms in the hydrogen bromide, giving radioactive hydrogen bromide. The radioactive bromine is recovered almost completely in this fo. m.

A large number of separations are made most conveniently by conventional chemical methods. In any such process, however, the danger of loss or contamination through coprecipitation should be considered. Impurities in the material bombarded may become radioactive, and accompany the desired active species during the separation. Much remains to be known regarding the behavior of matter at such small concentrations in passing through a series of reactions.

As an example, during the separation of radioactive zinc from copper, the precipitation of copper sulfide will result in some loss of zinc through adsorption on the precipitate. If no carrier is used for the zinc, such losses may account for an appreciable fraction of the activity. A satisfactory alternative method for this separation is either precipitation of the copper with salicylaldoxime (*16*) at a pH of about 3, or electrodeposition of the copper from sulfuric-nitric acid solution. The zinc remains in solution, and if no carrier is added, a high specific activity is obtained.

Electrochemical methods have been used in separations involving the radioelements. Electrodeposition from the gas phase or from solution has been attempted in the separation of radioiodine. Thus, when alkyl iodides are bombarded with neutrons, the corresponding radioactive isotope of iodine is formed. These active atoms are ejected from the molecule, as the result of gamma ray recoil, in charged form. Fermi (*15*) was unsuccessful in depositing radioiodine thus produced from gaseous ethyl iodide and methyl iodide. However, Paneth and Fay (*44*) succeeded in depositing iodine on copper and silver electrodes in good yield from neutron-irradiated liquid organic iodides. Govaerts (*19*) concentrated P^{32} after neutron bombardment of carbon disulfide by depositing the phosphorus directly from the carbon disulfide on a copper anode. Fay and Paneth (*13*) were able to deposit on copper and platinum electrodes no more than 35 per cent of the active arsenic produced by neutron bombardment of gaseous arsine.

Electrodeposition from aqueous solution is an effective means of separation. Radiocopper has been isolated by internal electrolysis on zinc, and also on a rotating lead plate (*24*). Steigman (*58*) used high-speed rotating disks and currents of 10 milliamperes for the same separation. Livingood and Seaborg (*36*) separated radioactive antimony by internal electrolysis on tin. Electrodepositions should prove of great value in making separations. Electrolysis with a mercury cathode (*25*) should be of use in many cases, although it does not appear to have been used as yet.

Extraction and adsorption methods for separating the radioelements have been successful. Grahame and Seaborg (*21*) have studied the partition of small amounts of radioelements between ether and hydrochloric acid, and conclude that extraction processes have certain unique advantages over other methods. The extracted element has a high specific activity, and the danger of contamination of the sample by some unsuspected radioelement is avoided. Data are given for the extraction of iron, gallium, and cobalt. Erbacher and Philipp (*11*) extracted radioactive halogens from neutron-irradiated alkyl halides with water.

These investigators also isolated radioactive halogens by adsorption on charcoal (*10*). The neutron-irradiated alkyl halides were shaken with charcoal, and the active halogen was later desorbed by boiling with water. Roginskiĭ and Gopstein (*49*) recommend the use of aluminum oxide as adsorbent. The chromatographic method thus may come to play an important part in making difficult separations. In separating radioactive gold Majer (*40*) irradiated with neutrons an alkaline gold chloride solution to which a small amount of colloidal gold had been added. The colloidal particles served as condensation nuclei for the radioactive gold which was formed, and as the colloidal gold flocculated the active gold was carried down with it. Erbacher (*8*) employed a somewhat similar method for concentrating radiophosphorus. White phosphorus was dissolved in carbon disulfide and the solution irradiated with neutrons. Some of the white phosphorus was converted into insoluble red phosphorus by gamma rays from the neutron source and the radioactive phosphorus (as phosphate ion) was adsorbed on the surface. The precipitate was merely heated in water to remove the radioactive phosphate.

By extraction with water Maier-Leibnitz (*39*) recovered radiophosphorus from neutron-irradiated triphenylphosphate dissolved in benzene. The yields were small. Better results were

secured by bombarding carbon disulfide with neutrons and filtering off the resulting small amount of precipitate which contained most of the active phosphorus.

Radioelements have also been separated by volatilization. Thus, Alvarez, Helmholz, and Nelson (*1*) volatilized radioactive cadmium from deuteron-bombarded silver. Ruben and Kamen (*52*) separated radioactive carbon from neutron-irradiated ammonium nitrate solution by pumping off the vapors, which contained the radiocarbon mainly as carbon dioxide.

From the foregoing survey, it appears that no fixed rules exist for separating radioelements in form suitable for use as indicators. In choosing a method, consideration must be given to the sharpness of the separation, the time required, and the form in which the active element is finally to be used. Many separations have been made merely for the purpose of establishing the chemical identity of some newly created radioelement, with no thought of indicator work. The splendid table of Seaborg (*53*) gives literature references which should lead to at least one method for separating any of the known radioelements. All the good methods of separation have not yet been discovered, however, and those interested in this field have ample opportunity to draw upon past experience and to exercise their ingenuity in developing new procedures.

Measurement of the Radiations

As the artificial radioelements are detected and determined by means of the ionizing radiations which they emit, the proper choice of apparatus for this purpose is of utmost importance. Three general types of detecting apparatus are in use at present. One simple and widely used type of detecting device is the quartz fiber (or Lauritsen) electroscope (*32*). This instrument is adapted to the measurement of all kinds of radiations emitted by the radioelements but has the disadvantage of being less sensitive than the other instruments.

A second somewhat more sensitive type is the combined ionization chamber–electrometer, which is also suitable for the detection of all kinds of radioactive radiations. A number of these instruments have been described (*3*, *7*).

The most sensitive detecting device obtainable, and at the same time probably the most useful for the chemist, is the Geiger-Müller counter, which may be adapted to the measurement of the activity of solids, liquids, or gases. Special types of counters for use with solutions have been constructed by Olson (*42*) and Bale (*2*), and for use with gases by Ruben (*51*) and Seaborg (*54*). A description of various counter circuits as well as the construction of several types of counters is given by Neher (*60*). For tracer studies the authors feel that the Geiger-Müller counter has wide applicability. In many cases the activity is diluted by the carrier, and again in the reaction itself. In adsorption experiments, for example, only small amounts of the radioelement are adsorbed, and the most sensitive means for measuring these quantities must be used. The type of counter used by Bale (*2*) for measuring the activity of liquids has proved extremely satisfactory in both chemical and biological studies.

If the radioactive element is merely to be detected in some phase of the reaction, the material has only to be placed somewhere near the counter tube or window of the ionization chamber, but this is insufficient for making quantitative measurements. When the radiation is used as a quantitative measure of an element, certain precautions are essential. The rate of decay of the material should be known. This can be found from published data, or better, from frequent measurements of the activity of the sample over a period of time. All measurements must be made in as nearly identical fashion as possible. The radioactive material should always be placed the same distance from the counter tube and the counter should always operate at the same voltage. A "background" count (count due to stray radiation) should always be taken and subtracted from the principal count. It is also desirable to check the counter for fluctuations by frequent counts on some long-lived natural radioactive material such as a potassium salt. Extremely strong sources should not be counted, for the counter generally is inaccurate above one thousand counts per minute. It is preferable to dilute the sample to give somewhat weaker activities. Strong samples are more conveniently measured on ionization chambers, where variation of the sensitivity is possible.

Occasionally the vessels used in making measurements on solutions become slightly radioactive.

Applications

Determination of Solubility. As it is possible to measure accurately extremely small amounts of a radioelement, radioactive indicators are helpful in making solubility measurements.

A definite amount of the insoluble compound is prepared containing a known amount of radioactivity. After equilibrium in the solvent has been reached, a portion of the solvent is removed and the activity measured. The fraction of the original activity recovered indicates the fraction of the material which dissolved. This method has been recently used in determining the solubility of ammonium phosphomolybdate (using radioactive phosphorus, *14*), cobaltic hydroxide (*5*), cobalt α-nitroso-β-naphthol and cobalt β-nitroso-α-naphthol (*4*). In the case of cobaltic hydroxide, the value found for the solubility was 5.6×10^{-3} mg. per liter, as contrasted with an earlier value of 3.18 mg. per liter obtained by another method. Presumably the higher value was the result of colloidal cobaltic hydroxide remaining in the solution. The solubility of cobalt α-nitroso-β-naphthol was found to be 1.5 mg. per liter, while the β-nitroso-α-naphthol derivative dissolved to the extent of only 0.17 mg. per liter. Ishibashi and Kishi (*27*) determined the solubility of lead salicylaldoxime using thorium B (an isotope of lead) as indicator and obtained the value 4.0×10^{-6} mole per liter.

Efficiency of Separation. One of the earliest studies of this type was made by Erbacher and Philipp (*9*). Using radioactive gold, they studied the completeness with which gold can be separated from platinum and iridium in mixtures containing the three elements.

When a hot alkaline solution of the elements was treated with sodium formate, the metals were precipitated. After washing and igniting, they were digested with aqua regia, whereupon the gold and platinum dissolved, leaving the iridium behind. The results obtained for iridium by this procedure were always high, presumably indicating incomplete removal of gold and platinum. Examination of the iridium fraction indicated that the gold (radioactive) was incompletely removed.

The solution containing gold and platinum, after separation from iridium, was treated with hydrogen peroxide, which precipitated the gold. When precipitation was thought complete, the gold was filtered off and platinum precipitated from the filtrate with sodium formate. In the case of the gold fraction, high results again were obtained, presumably the result of contamination by platinum. Measurement of the activity revealed, however, that contamination by platinum was greater even than had been expected. Actually, only 97 per cent of the gold was recovered in the so-called gold fraction. The remaining 3 per cent appeared in the platinum fraction. In another series of experiments, it was found that even after half of the platinum had been precipitated along with the gold by the peroxide, recovery of the gold was incomplete. The unsatisfactory nature of the peroxide separation was thus demonstrated.

In connection with other studies, Wiig and Flagg (*65*) found the precipitation of stannous tin by potassium ferricyanide to be complete in solutions 1 molar in hydrochloric acid. Radioactive tin was produced by deuteron bombardment of metallic tin. This was dissolved in hydrochloric acid, and aliquot parts of the solution were precipitated with excess ferricyanide. Examination of the filtrate with a counter of the type described by Bale (*2*) indicated complete removal of the tin.

The work of Grahame and Seaborg (*21*) indicates another aspect of analytical chemistry which is served by radioactive indicators. The extraction of iron and gallium chlorides by ether was shown to be extremely efficient as a means of separating these elements from cadmium and manganese. A method was also suggested for the identification of radioelements, using the extraction procedure. The distribution coefficient for a known stable element between two phases may be determined. Then, if an unknown radioelement is found, its distribution coefficient may be determined, which in turn serves for its identification.

Coprecipitation. Studies of a somewhat different nature are being carried out by Flewelling and Flagg (*17*). Using radioactive beryllium, the behavior of this element in separations made with 8-hydroxyquinoline is being studied. Thus, for example, it has been found that in the separation of aluminum and beryllium by the method of Kolthoff and Sandell (*30*), the pH must be kept below a value of 6. At higher pH values the appearance of radioactivity in the precipitate of aluminum hydroxyquinolate indicates that beryllium is also being precipitated.

Properties of Analytical Precipitates. An extension of the method of Paneth (*43*) for studying the properties of surfaces, hitherto confined to elements having natural radioactive isotopes, has become possible by use of artificial radioactive indicators. Thus, the specific surface of silver chloride has been determined (*31*) by shaking the precipitate in a solution containing radioactive chloride ions and measuring the rate of uptake of the radioactive ions.

Kolthoff and O'Brien (*29*) have measured the specific surface of silver bromide by the method of Paneth, using radioactive bromine. They found that exchange between bromide ion and silver bromide was not confined to the surface layer. Only in the presence of a dye such as wool violet, which prevented the recrystallization process from taking place, could exchange be confined to the surface layer of the precipitate. On surfaces containing wool violet, however, it was possible to measure the specific surface of both fresh and aged precipitates. They found that freshly precipitated silver bromide had a large surface, and that the air-dried product underwent thermal aging. Such an aging process requires that silver bromide on an active (fresh) surface have marked thermal mobility, and that ions or molecules can move around to more normal positions. When fresh surface is continuously exposed as a result of such a process, there should be an opportunity for the exchange $AgBr + BrBr^* \leftrightarrows AgBr^* + BrBr$ to take place. It was found that upon shaking the solid with radioactive bromine either dissolved in ethyl bromide or in the gas phase, the speed of incorporation of radioactive bromine into the solid decreased as the surface decreased and that the radioactive bromine actually penetrated the solid below the surface. Thus the mechanism of the thermal aging process was shown.

Somewhat similar experiments have been performed by Polesitskiĭ (*46*), who attributed a decreasing rate of exchange between bromide ion and solid silver bromide to the aging process. Preaged precipitates gave slower rates than did fresh precipitates, and silver bromide which had been previously fused showed no exchange.

Analytical Methods for Rare Elements. Artificial radioactivity promises to be extremely useful in extending our knowledge of the analytical chemistry of the rarer elements. At the time of the discovery of the artificial radioelements, numbers 43, 61, 85, and 87 were still unknown or in doubt. Elements 43, 85, and probably 61 (*47*) have been prepared artificially and the behavior of 43 and 85 has been investigated with the aid of related stable elements as carriers. Element 85 has been made by bombarding bismuth with 32 M. e. v. alpha particles and its properties have been studied by Segré, MacKenzie, and Corson (*57*). Using iodine as a carrier, 85 was found to be much more metallic that iodine and to bear little resemblance to the halogens. It is precipitated by hydrogen sulfide but not by silver nitrate; it is extracted by carbon tetrachloride to a much smaller extent than is iodine, etc. Past attempts to isolate 85, based on a strict similarity with iodine, probably failed because of such differences in behavior.

Similarly, radioelement 43 has been found (*45*, *56*) to resemble rhenium in not being precipitated along with manganese-sulfide and to differ from rhenium in not being volatilized from a hot sulfuric acid solution in a current of hydrogen chloride. The discovery of such differences in behavior, together with the use of the artificial radioelement as a tracer, should lead to methods of concentration and analysis of stable isotopes of elements 43, 85, 93, etc., should they exist in detectable quantities.

Analysis by "Radioactivation". One very interesting application of radioelements is the determination of traces of impurity in a substance by conversion of the impurity into a radioactive substance by bombardment in the cyclotron.

In order to test for gallium in pure iron, Seaborg and Livingood (*55*) bombarded a sample with deuterons. When pure iron is bombarded with deuterons the reaction Fe^{58} (d,p) Fe^{59} is the only one giving rise to radioactivity. If present, Ga^{69} and Ga^{71} would yield radioactive Ga^{70} and Ga^{72}. For the sake of simplicity, let us consider only Ga^{72}, though both must be taken into account. Thus, when iron containing gallium is bombarded with deuterons, radio-Fe^{59} and radio-Ga^{72} result. These two radioelements have half-lives of 47 days and 14 hours, respectively, and so the radioactive intensities in the bombarded sample were readily determined to be in the ratio of 2000 to 1. If the quantities of Fe^{58} and of Ga^{71} in the iron sample are the same, and if the efficiencies of conversion (or the yield) are the same, then the radioactive intensities should be the same for the same bombardments. For two elements as close in atomic number as iron and gallium, the efficiencies may be assumed to be the same, so that the activities depend only on the relative amounts of Fe^{58} and Ga^{71} present. Ordinary iron contains only 0.28 per cent Fe^{58} and since the activity of Ga^{72} is $^1/_{2000}$ that of Fe^{59}, the amount of Ga^{71} must be $^1/_{2000}$ that of Fe^{58}. It was found that the sample of iron contained about 6 p. p. m. of gallium, and one tenth of this quantity could readily have been detected. Similar experiments have been carried out on copper in silver (*28*), copper in nickel (*55*), iron in cobalt oxide (*55*), sulfur in a sheet of paper (*55*), and dysprosium in fractions of yttrium earths (*18*).

In using activation as a method of analysis great care must be taken to avoid introduction of traces of impurities during the bombardment. While this procedure will probably be used only infrequently, it illustrates the power of this new tool.

Stable Isotopes

Since this review deals primarily with the radioelements as tracers, only brief mention will be made of the use of stable isotopes. For a discussion of deuterium the reader is referred to the book by Farkas (*12*). Through the efforts of Urey and his colleagues (*26*, *59*, *62*) limited quantities of enriched samples of other stable isotopes, notably C^{13}, N^{15}, and S^{34} are available at present with commercial production soon to be realized.

A sample containing more than the natural or normal percentage of the desired isotope (C^{13}, etc.) is in general obtained by (1) diffusion through porous membranes or a gaseous substance, (2) the thermal diffusion method of Clusius and Dickel, or (3) exchange reactions and distillations which produce differences in composition by taking advantage of differences in physical and chemical properties. Except for deuterium, the most convenient method of quantitatively

determining the amount of the heavy isotope present is by the mass spectrometer. The construction, operation, and cost of a suitable instrument have been discussed in detail by Nier (*41*).

Having a sample enriched in the isotope of an element, the sort of use to which it can be put is well illustrated by the excellent work of Rittenberg and Foster (*48*, *63*) on the analysis of mixtures of amino acids.

From such a mixture of acids one can in general isolate either all of a particular amino acid in impure form or some of it in a pure state, but not all of the pure acid. The per cent of the pure acid can be determined, however, by the "isotope dilution method". The procedure is to add a grams of pure amino acid A containing C_0 atom per cent excess of N^{15} (or C^{13}) to a known weight, W, of a mixture of amino acids and then to isolate from this mixture a pure sample of amino acid A. (Obviously the a grams added become uniformly mixed with that already present in the unknown mixture.) The pure sample of amino acid A just isolated is now analyzed for N^{15}, by the mass spectrometer.

If it is found to contain C atom per cent excess of N^{15}, then the fraction of the amino acid A present in the original unknown mixture is given by $(C_0/C - 1)\frac{a}{W}$. Since only very small samples are needed for an isotope analysis, large losses in purification are permissible. According to Rittenberg, the error of the method may be reduced to 1 per cent by the proper choice of conditions.

Obviously, this method may be applied to the analysis of other mixtures, provided there is no loss of the heavy isotope through exchange reactions and provided a pure sample of the substance, for which analysis is being made, can be isolated. It is claimed that the method can be modified so that the isolated sample need not be quite pure (*20*).

Conclusion

The methods outlined have, of course, a number of advantages and disadvantages as compared with the usual analytical procedures. Tracer isotopes can be used to solve many problems with which ordinary methods cannot possibly cope. The analysis can often be performed more rapidly and more conveniently, particularly with radioactive isotopes, than by ordinary qualitative and quantitative analysis. Furthermore, there is no doubt about the identity of the substance being measured—that is, only one species can be radioactive or exhibit the proper half-life period. Finally, the accuracy of a determination can be varied by counting much or little, as desired (for a discussion of errors in counting, see Neher, *60*).

One disadvantage inherent in the use of either stable or radioactive isotopes is the lack of general availability of the isotopes themselves and of the necessary instruments for their quantitative determination. These substances are, however, becoming increasingly available and the detection devices are not expensive to build and operate. As compared with the counter the mass spectrometer is somewhat more expensive both in construction and operation. The reproducibility of results is perhaps not so great as with standardized analytical procedures but with exercise of reasonable care satisfactory accuracy can be attained.

In making use of tracer analysis, a choice may have to be made between the use of a stable or radioactive isotope, as with carbon, nitrogen, or sulfur. Here a number of facts will have to be considered, such as availability of the isotope and the measuring device, the relative costs of the two procedures, the time available for an experiment (the only radionitrogens known are N^{13} and N^{16} with half-lives of 10 minutes and 8 seconds, respectively), and the extent to which the tracer atom can be diluted and still be determined quantitatively. Like any other method, the isotope method cannot be used indiscriminately in attacking chemical problems. Its success in the hands of any investigator will be determined by the care and thoughtfulness with which it is used.

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The S. I. L. Viscometer

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A kinematic viscometer has been developed and proved practical in actual routine testing operations. It is almost, if not fully, as satisfactory as a more expensive patented instrument of foreign origin which has been extensively used.

DURING the past few years there has been an increasing tendency in laboratories of the petroleum industry to determine viscosity by means of long-capillary glass instruments, commonly designated as "kinematic" viscometers. These instruments are fundamentally more accurate than short-outlet "commercial" viscometers such as the Saybolt, Redwood, and Engler, and have been used for many years in research or investigative work. They are now being adopted for routine testing and considerable progress has been made in minimizing certain inherent deficiencies, such as fragility and the need for fussy manipulation.

The two kinematic viscometers which are most familiar to the petroleum industry in the United States are probably the Ubbelohde (*5*) and the Fenske (*2*). The first is so costly that its use has been restricted and the second lacks several characteristics which the authors consider desirable. The Zeitfuchs (*6*) viscometer possesses fundamental characteristics which seem to place it almost on a par with the Ubbelohde, but it is also patented and the original negotiations for its use by the companies with which the authors are associated were unsuccessful. The work of developing the S. I. L. viscometer, described in this paper, had reached an advanced stage before the dedication of the Zeitfuchs' patent to the public was announced. The S. I. L. instrument, as finally evolved, differs from the Zeitfuchs sufficiently to justify a published description.

Characteristics of Viscometers

The U-tube type of viscometer was described by Ostwald (*4*) about 1893 and has been used in numerous forms and modifications. The B. S. I. (*3*) U-tube viscometers are standards of the Institute of Petroleum of Great Britain. They are of the conventional Ostwald type and do not seem to involve any original features.

The Fenske (*1*, *2*) modification of the Ostwald viscometer is characterized by a bent form which minimizes errors due to deviations from a strictly vertical position while in use. This advantage is attained by some sacrifice of precision in filling, and it is necessary to use calibration constants that vary with the temperature of test. The Fenske viscometer is, however, widely used in the United States and is a practical instrument in spite of its obvious defects.

The Ubbelohde (*5*) or suspended level viscometer represents an important advance over the conventional Ostwald or U-tube type. Its characteristic feature from the point of view of routine use is freedom from the necessity of accurate filling. There are numerous other theoretical and practical advantages, including the possibility of construction with multiple capillaries as proposed by FitzSimons (*1*).

The Zeitfuchs (*6*) viscometer is an ingenious development and the details adapting the instrument to routine use have been worked out in a thoroughgoing manner. The characteristic feature is an overflow gallery and auxiliary withdrawal tube on the capillary arm, which facilitates precise nonautomatic adjustment of the volume of oil.

The S. I. L. viscometer, described in this paper, embodies the feature of an overflow gallery which is on the open arm instead of the capillary arm and which permits precise automatic establishment of the correct volume after the oil under test has reached the desired temperature. This overflow gallery has a definite similarity to that of the familiar Saybolt viscometer.

Description

Figure 1 and Table I indicate the form and dimensions of the series of S. I. L. viscometers at present in use. The dimensional tolerances have been accepted as practical manufacturing specifications by the commercial organization from which instruments now in use have been purchased.

CALIBRATION. Instruments, as purchased, are uncalibrated and lack the upper circular mark (4, Figure 1). Test runs are made in comparison with previously standardized viscometers and the position of the mark is established so that constant C (Equation 1 or 2) is within ± 0.2 per cent of the standard value for the size of instrument under test. The mark is then engraved on the glass by the conventional acid etching method.

OPERATION. With the upper end of tube 2 (Figure 1) closed by the operator's fingertip, the liquid to be tested is poured into tube 1 and allowed to flow through opening J until this is submerged at least 0.62 cm. (0.25 inch), but not more than 1.9 cm. (0.75 inch), with bulb A completely filled. The liquid should be free from extraneous matter and preliminary filtration or straining is frequently desirable. It can be poured directly into the viscometer from a lipped beaker but if spillage is likely to cause inconvenience, a small funnel may be used. If the liquid does not flow readily through J, it may be warmed moderately. This is rarely necessary except when extremely viscous products are to be tested at temperatures appreciably above 38° C. (100° F.).

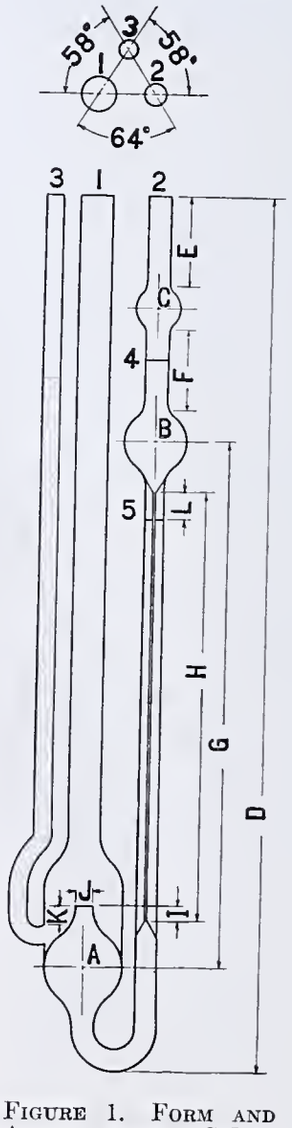

FIGURE 1. FORM AND ARRANGEMENT OF S. I. L. VISCOMETER

After introducing the liquid the upper end of tube 2 is opened, and the filled viscometer is placed in the thermostatic bath. Sufficient time is allowed to bring the liquid to the temperature of test and the excess is then withdrawn through tube 3 by suction (preferably mechanical suction with a trap in the line to retain the fluid). Suction is then applied to tube 2 and the liquid is drawn up until its upper level is just below the middle of bulb C. Particular care must be taken at the beginning of this operation to avoid even the slightest positive pressure in tube 2, as it will cause some of the liquid to pass through orifice J into gallery K and destroy the accuracy of the volume adjustment previously made.

The suction is then released and the time required for the meniscus to pass from mark 4 down to mark 5 is determined. The liquid is then brought up into C again and the timing operation repeated. It is usually desirable to make at least three deter-

minations and a larger number is necessary if the difference between any two values is in excess of 0.1 per cent. It is desirable to have a series of determinations with the average difference from the mean not in excess of 0.05 per cent. The instrument is then emptied, cleaned, and dried, preliminary to use with another sample. Except for referee testing, check determinations involving a refilling with the same sample are rarely necessary unless there is uncertainty regarding the homogeneity of the liquid or the cleanliness of the apparatus.

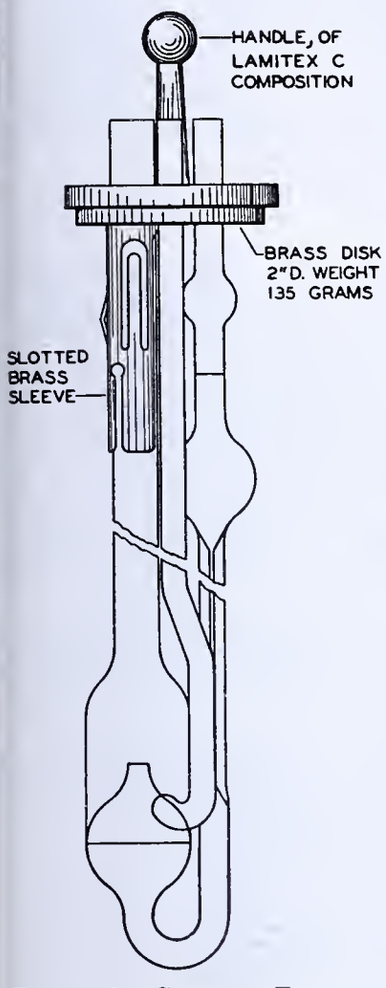

FIGURE 2. GENERAL FEATURES OF TUBE HOLDER

Accessories

There are a number of major and minor accessories that are essential for accurate and efficient viscosity measurement. These range from thermometers and thermostatic baths to assemblies of tubing and cocks for applying suction and pressure. The authors and their associates have developed a number of these accessories which have been found satisfactory. Most of them, however, are of general rather than specific usefulness and it is not proposed to include a detailed description in the present paper.

Two accessories, however, were developed to take care of specific requirements of the Ubbelohde and S. I. L. viscometers.

TUBE HOLDER. The tube holder shown in Figure 2 was developed to accomplish accurate alignment of the viscometer in a vertical position, easy removal from the thermostatic bath and easy separation of the holder and the viscometer, and protection of the viscometer from breakage.

The tube holder is usable only with thermostatic baths having rigid top plates and accurately machined recess surfaces on which the holders rest. The circular form shown in Figure 2 is desirable because it is both easy to fabricate and convenient for the operator, who can rotate the holder to the position which suits him best without changing its alignment from the vertical. A square or rectangular form is, however, entirely usable. It is desirable to have holders interchangeable and this necessitates standard sizes and spacing for the holes and accurate adherence to the dimensional limits specified for the viscometers.

Other types of holders accomplishing the same results can undoubtedly be made and the present description is offered simply as a report on a mechanical development that has proved highly satisfactory and has taken care of the varying preferences concerning such details as filling, operating, and cleaning viscometers with or without intermittent removal from thermostatic baths. The holder, when in place, provides a degree of protection against breakage equivalent to that of the metal supporting member of Zeitfuchs or a glass bridge such as is usually found on Fenske viscometers. The construction of the holder is such that glass bridges could be used on S. I. L. or Ubbelohde viscometers, if desired, to reduce the possibility of breakage when instruments are not in the holders. In addition, it would be entirely feasible to attach a shielding cage which would cover the entire length of the viscometer. Operators associated with the authors have indicated a preference for instruments without bridges and the breakage has been so infrequent that cages have not seemed necessary.

CLEANING RACK. The S. I. L. viscometer is slightly inferior to most if not all of the other types of kinematic instruments with respect to ease of cleaning be-

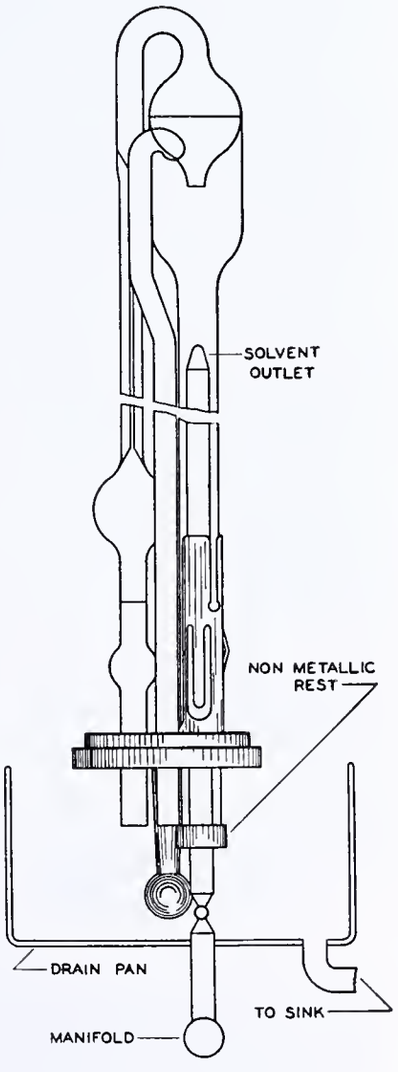

FIGURE 3. DETAILS OF CLEANING RACK

TABLE I. DIMENSIONS OF S. I. L. VISCOMETERS

(Refer to Figure 1)

		Mm.
Outside diameter		
Bulb *A*	Approx.	26
Bulb *B*	Approx.	21
Bulb *C*	Approx.	13
Tube 1		11 ± 0.2
Tube 2	Approx.	7
Tube 3	Approx.	6
Center distance		
From 1 to 3		17 ± 0.3
From 1 to 2		18 ± 0.3
From 2 to 3		17 ± 0.3
D		295 ± 3.0
E		40 minimum
F		25 minimum
G		178 ± 2.0
H		145 ± 0.5
I		5 ± 1.0
K		7 minimum
L		20 ± 2.0
		Ml.
Volume of *A*		7 minimum
Volume of *B*		4.8 ± 0.1

S. I. L. Viscometer Size No.	Calibration Constant, *C*	Capillary Diameter, Mm.	Inside Diameter of Opening *J*, Mm.
1	0.01	0.62 ± 0.01	4.5 ± 0.1
2	0.10	1.10 ± 0.01	5.5 ± 0.1
3	1.00	1.96 ± 0.02	6.5 ± 0.1
4	10.00	3.50 ± 0.02	7.5 ± 0.1

Wall thicknesses of tubes 1, 2, and 3, approximately 1.0 mm.
Capillary, trumpet-shaped ends
Marking, size number and manufacturer's or purchaser's serial number etched or otherwise permanently marked, preferably on flare at lower end of tube 1

tween tests. This is due to the gallery. The Zeitfuchs viscometer also has a gallery but if it is not cleaned perfectly no harm results, whereas any unremoved liquid in the gallery of the S. I. L. instrument may contaminate the oil under test.

The cleaning rack shown in Figures 3 and 4 was actually developed to facilitate operations with Ubbelohde viscometers but is particularly valuable with the S. I. L. instrument. It is so convenient and efficient that operators using it do not notice any important difference between the two instruments with respect to cleaning.

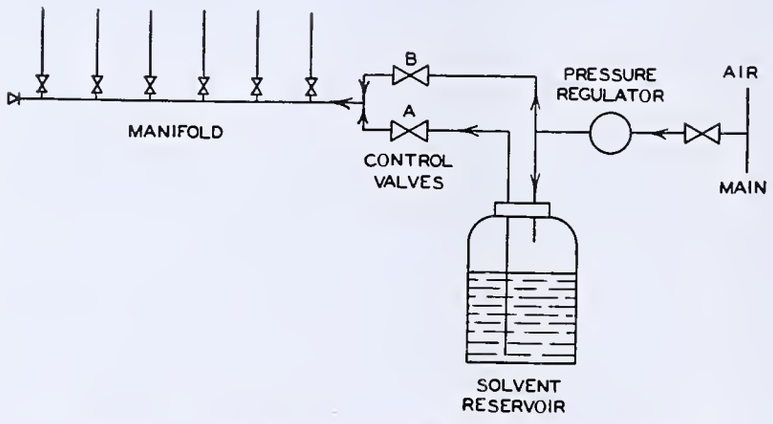

FIGURE 4. GENERAL ARRANGEMENT OF CLEANING RACK

The actual cleaning procedure involves placing a viscometer, drained reasonably free of oil, in the position indicated. The holder need not be removed. Valve A (Figure 4) is opened for 1 or 2 seconds, and a small quantity of solvent is blown in. This is allowed to drain briefly and the operation is repeated several times. The viscometer is moved around so that some of the solvent is injected through opening J (Figure 1) and washes out the capillary arm of the instrument. After washing is completed, which may in extreme cases require eight or ten "squirts" of solvent, valve A is closed and valve B is opened. Air is blown through the viscometer until it is dry, which usually requires only a minute, although under ordinary operating condition, no efficiency is lost by letting instruments remain "on the air" for a considerably longer period.

The preferred solvent in the authors' laboratory is a mixture of about 60 per cent carbon tetrachloride and 40 per cent of a petroleum naphtha having an initial boiling point of about 38° C. (100° F.) and a final boiling point of about 150° C. (300° F.). By installing the drying rack in a location where there is no fire hazard it would be possible to use straight petroleum naphtha. The solvent injected and the air used for drying are under a gage pressure of 350 to 700 grams per sq. cm. (5 to 10 pounds).

Figure 3 shows a single section of the rack. The usual construction is a six-tube installation, as shown diagrammatically in Figure 4, and an expert operator can clean and dry the full number of viscometers in from 5 to 10 minutes. The consumption of solvent need not be in excess of 400 cc. per tube cleaned.

Calibration Constants

The conventional equation for representing the relationship between kinematic viscosity and efflux time is:

$$V = Ct - \frac{B}{t} \qquad (1)$$

where V = kinematic viscosity in centistokes
t = outflow time in seconds
C = so-called calibration constant of the instrument
B = so-called kinetic energy constant of the instrument

The method of calibrating S. I. L. viscometers so that constant C will be a predetermined value (0.01, 0.1, 1.0, or 10.0) has been described above. Constant B varies with individual instruments and must be actually determined by suitable methods if the highest degree of precision is required.

An adequate degree of precision is possible for all oils having viscosities in excess of 3 or 4 centistokes by assumin that the average B-constant for S. I. L. viscometers is 0.7 This figure was obtained by Zeitfuchs (*6*) for instrument of the same essential dimensions, and although questionabl from a theoretical point of view has proved reasonabl close to the limited number of actual values determined b the authors through use of the A. S. T. M. (*1*) method.

When the magnitude of the term $\frac{B}{t}$ is negligible in compar son to the term Ct, Equation 1 may be reduced to the simple form

$$V = Ct \qquad (2)$$

By following the A. S. T. M. practice (*1*) of avoidin efflux times less than 80 seconds it is possible to dispens with kinetic energy corrections for the No. 3 and No. instruments and also for the No. 2 unless errors of le than 0.1 per cent must be avoided.

With the No. 1 S. I. L. viscometer the kinetic energ correction, if $B = 0.77$, is about 0.1 per cent whe the efflux time is 275 seconds (equivalent to 2.7 centistokes). When testing liquids of lower viscosity suitable allowances should be made for kinetic energy If high accuracy is essential, the B-constant for the ind vidual instrument must be known and the results com puted by the use of Equation 1. The use of correctio values shown in Table II is convenient if moderat accuracy is adequate.

TABLE II. NEGATIVE CORRECTIONS FOR VARIOUS OUTFLO TIMES WITH NO. 1 S. I. L. VISCOMETER

Efflux Time *Seconds*	Correction *Centistoke*	Efflux Time *Seconds*	Correction *Centistoke*
80–89	0.010	180–189	0.004
90–99	0.009	190–199	0.004
100–109	0.008	200–209	0.004
110–119	0.007	210–219	0.004
120–129	0.006	220–229	0.004
130–139	0.006	230–239	0.003
140–149	0.006	240–249	0.003
150–159	0.005	250–259	0.003
160–169	0.005	260–269	0.003
170–179	0.005	270–279[a]	0.003

[a] Above 280 no correction unless an accuracy in hundredths of a per cen is expected.

S. I. L. Viscometers of Other Dimensions

The possible usefulness of viscometers having C constant intermediate between those of the series described abov has been recognized. Experimental instruments are bein obtained and it is planned to give them a trial in actua testing service.

Commercial Availability

The authors have been able to purchase uncalibrate S. I. L. viscometers made to the specifications listed i Table I at less than two thirds of the price they pay cur rently for uncalibrated Ubbelohde instruments supplied b the same manufacturer. The amount of laboratory worl involved in adjusting S. I. L. viscometers to predetermine calibration constants is only slightly more than the work o evaluating constants for tubes already marked.

No commercial supply of reliably adjusted or calibrate viscometers has as yet been developed, nor is any know to exist for the other types of "kinematic" instruments. I is believed that this deficiency may be corrected whe kinematic viscometers are used by a larger number of labora tories that are not equipped to do their own calibration work

The cleaning rack described is commercially available.

Acknowledgments

W. H. Schaer, instrument maker of the Standard Inspecon Laboratory, deserves credit for the development of the ıbe holder described in this paper. The cooperation of ıe commercial organizations manufacturing the viscometers ıd the cleaning racks has been very helpful.

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Quantitative Determination and Separation of Copper with Benzotriazole

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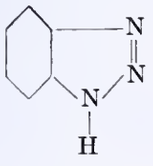
A new organic precipitant for copper, benzotriazole, is described. Copper is precipitated in a tartaric-acetic acid solution at a pH of 7.0 to 8.5. In the absence of silver, nickel, cadmium, zinc, cobalt, and ferrous iron the precipitate can be weighed directly. In their presence the reagent can be used to effect a preliminary separation, and the copper subsequently determined by a standard method—for example, electrolytically or by the potassium iodide–thiosulfate method.

Benzotriazole is an excellent precipitant for copper in iron and steel preparatory to the analysis by the standard iodide method. It possesses the advantage over the sulfide precipitation that arsenic, antimony, and molybdenum are not precipitated.

The paper presents data on the proper conditions of acidity, temperature, pH, etc., for precipitation. A detailed procedure for determining copper in iron and steel is given.

ALTHOUGH benzo-1,2,3-triazole



ıas been known for many years, little work on its application o the quantitative analysis for copper has been published. Remington and Moyer (*4*) made a qualitative study of the reıgent and also reported a gravimetric procedure for the quantiative determination of cuprous copper, silver, and zinc.

The compound itself is light, white, needle-crystalline, and oluble in alcohol, benzene, and water, a 2 per cent aqueous solution at room temperature being nearly saturated. It reacts with copper in ammoniacal solution to give a blue-green precipitate of the composition $(C_6H_4N_3)_2Cu$. Experimental work was undertaken in the chemical laboratory of the Battelle Memorial Institute to determine the conditions under which the precipitation of copper is complete, particularly in the analysis of copper in cast irons, steels, and other copper-bearing materials.

The advantages of a specific reagent to precipitate metals are known to all analytical chemists. Benzotriazole, as shown in this paper, is specific for copper, but certain other ions are also precipitated. In the presence of Fe^{++}, Ag^{+}, Ni^{++}, Cd^{++}, Zn^{++}, and Co^{++} the copper precipitate cannot be weighed directly. On the other hand, the copper can be precipitated from a solution containing arsenic, antimony, molybdenum, and tin which are coprecipitated with copper by hydrogen sulfide and which interfere in subsequent potassium iodide–thiosulfate determinations.

While some investigators (*1*, *2*, *3*) have shown that copper can be titrated in the presence of moderate amounts of arsenic, trivalent iron, and antimony by the addition of fluorides, phthalates, and acetates as buffers, the presence of large amounts of these elements is still undesirable. Park (*3*) states that the presence of large amounts of fluoride reduces the accuracy of the titration and that if manganese is present with iron and arsenic the solution is buffered. In any event, where speed and accuracy are required, the absence of interfering elements is highly beneficial, as no special time-consuming treatments are then necessary.

The benzotriazole method, therefore, can be used for the direct precipitation and gravimetric determination of copper in the absence of certain interfering elements. In the presence of interfering ions the reagent may be used to effect a preliminary separation and the copper in the copper triazole precipitate can be determined iodometrically, electrolytically, or by other standard procedures.

Experimental Work

The experimental results on establishing the best conditions to precipitate the copper are reported below. Precipitation is done in a solution containing tartaric and acetic acid. In most of the experimental work the copper in the copper triazole precipitate was determined by the potassium iodide–

TABLE I. EFFECT OF AMOUNT OF BENZOTRIAZOLE ON PRECIPITATION OF COPPER

Benzotriazole Added[a]	Copper Present	Copper Found	Difference
Ml.	*Gram*	*Gram*	*Gram*
15	0.0101	0.0015	−0.0086
25	0.0101	0.0035	−0.0066
35	0.0101	0.0090	−0.0011
45	0.0101	0.0100	−0.0001
55	0.0101	0.0102	+0.0001
65	0.0101	0.0101	0.0000
50	0.0101	0.0102	+0.0001
50	0.0101	0.0101	0.0000
50	0.0180	0.0180	0.0000
50	0.0172	0.0173	+0.0001

[a] 2 per cent aqueous solution.

TABLE II. EFFECT OF TEMPERATURE AND TIME OF DIGESTION ON PRECIPITATION OF COPPER WITH BENZOTRIAZOLE

Temperature	Time of Digestion	Copper Present	Copper Found	Difference
° *C.*	*Min.*	*Gram*	*Gram*	*Gram*
100	30	0.0101	0.0101	0.0000
100	15	0.0101	0.0100	−0.0001
100	5	0.0101	0.0101	0.0000
25	30	0.0101	0.0101	0.0000
25	15	0.0101	0.0101	0.0000
25	5	0.0101	0.0100	−0.0001

thiosulfate method. In this connection it was found that the precipitate must be ignited to cupric oxide. If it is merely dissolved in nitric acid, the copper triazole will again precipitate when the solution is neutralized and made acid with acetic acid prior to the addition of potassium iodide.

HYDROGEN-ION CONCENTRATION REQUIRED FOR COMPLETE PRECIPITATION OF COPPER. Copper was precipitated by benzotriazole from a series of synthetic copper solutions containing known amounts of copper, and also containing tartaric acid and acetic acid to approximate the conditions under which the copper would ordinarily be precipitated in an analysis. The solutions were adjusted to the desired pH by adding ammonia, using a glass-electrode type of pH meter, and the copper was precipitated with benzotriazole. The precipitate was then filtered, ignited to copper oxide, and titrated by the potassium iodide–thiosulfate method. The precipitation was complete between pH 7.0 and 8.5. Below pH 5.0 no precipitation occurred, while at pH 8.8 the precipitate began to dissolve.

AMOUNT OF BENZOTRIAZOLE NECESSARY FOR COMPLETE PRECIPITATION OF COPPER. The amount of reagent necessary for the complete precipitation of copper from ammoniacal tartrate solution was determined by adding increasing amounts of a 2 per cent aqueous solution of benzotriazole to synthetic solutions of copper that had been adjusted to the proper hydrogen-ion concentration. The results in Table I indicate that at least 45 ml. of the reagent solution are required to precipitate completely 0.0100 gram of copper, and that 50 ml. of the reagent solution effect the complete separation of 0.0180 gram of copper. This latter amount represents the upper limit of copper permissible for rapid and convenient handling of the voluminous precipitate.

EFFECT OF TEMPERATURE AND TIME OF DIGESTION. Results shown in Table II indicate that a temperature of 25° to 100° C. and a digestion time of 5 to 30 minutes were equally effective in quantitatively precipitating the copper. As an aid to filtration, however, it is recommended that the solution be kept hot (70° to 80° C.) and that it be digested until the precipitate fully coagulates.

EFFECT OF VOLUME. Table III shows that the volume of the solution, within comparatively wide limits, is without effect on the completeness of precipitation of the copper.

SENSITIVITY OF PRECIPITATION OF COPPER BY BENZOTRIAZOLE. The sensitivity of the copper-benzotriazole reaction was determined by adding benzotriazole to solutions containing known amounts of copper. The copper solutions were 200 ml. in volume, containing 7 grams of tartaric acid and 10 ml. of acetic acid, and were adjusted with ammonium hydroxide to the litmus color change. The results, shown in Table IV, indicate that as little as 0.0002 gram of copper is determinable in 200 ml. of solution.

PERCENTAGE OF COPPER IN COPPER TRIAZOLE PRECIPITATE. In order to determine the percentage of copper in the copper triazole precipitate, synthetic copper solutions were precipitated by benzotriazole and the precipitate was filtered on weighed sintered-glass filtering crucibles. The crucible and precipitate were dried for 2 to 3 hours at 135° to 140° C. cooled in a desiccator, and weighed. Temperatures of 105° to 110° C. were found to be too low for satisfactory drying.

TABLE III. EFFECT OF VOLUME OF SOLUTION ON PRECIPITATION OF COPPER WITH BENZOTRIAZOLE

Volume of Solution	Copper Present	Copper Found	Difference
Ml.	*Gram*	*Gram*	*Gram*
125	0.0101	0.0101	0.0000
200	0.0101	0.0101	0.0000
300	0.0101	0.0101	0.0000
500	0.0101	0.0101	0.0000

TABLE IV. ANALYSES OF STANDARD COPPER SOLUTIONS BY BENZOTRIAZOLE IN A VOLUME OF 200 ML.

Copper Present	Copper Found	Difference
Gram	*Gram*	*Gram*
0.0002	0.0002	0.0000
0.0005	0.0005	0.0000
0.0007	0.0007	0.0000
0.0010	0.0010	0.0000
0.0020	0.0020	0.0000
0.0038	0.0038	0.0000
0.0051	0.0051	0.0000
0.0089	0.0089	0.0000
0.0100	0.0100	0.0000

The results in Table V indicate that the copper triazole precipitate is of the formula $(C_6H_4N_3)_2Cu$ and the copper found in the precipitate is in close agreement with the theoretical 21.216 per cent. Except in the presence of interfering elements, such as those discussed in the following paragraph, the direct gravimetric determination of copper as $(C_6H_4N_3)_2Cu$ is possible.

ELEMENTS PRECIPITATED BY BENZOTRIAZOLE IN AMMONIACAL TARTRATE SOLUTION. Qualitative tests showed that benzotriazole precipitates the following metals, either completely or partially, from ammoniacal tartrate-acetate solution: Fe^{++}, Ag^{+}, Ni^{++}, Cd^{++}, Zn^{++}, and Co^{++}. The following are not precipitated: Fe^{+++}, Se^{++++}, Te^{++++}, Sb^{+++}, Sb^{+++++}, As^{+++++}, Sn^{++}, Cr^{+++}, Cr^{++++++}, Al^{+++}, and Mo^{++++++}.

RECOMMENDED PROCEDURE FOR COPPER IN CAST IRONS AND STEELS. Inasmuch as interfering elements are usually present, the direct gravimetric method is not applicable to irons and steels. The benzotriazole method, however, separates the copper from such elements as arsenic, antimony, molybdenum, and tin which interfere in the hydrogen sulfide–potassium iodide–thiosulfate method. Benzotriazole is used for the preliminary separation, and the copper can be determined in the precipitate by standard methods. The writer recommends the iodide-thiosulfate method because of its applicability, speed, and simplicity.

Table V. Percentage of Copper in Copper Triazole Precipitate

Copper Added	Copper Triazole Precipitate	Copper in Triazole Precipitate
Gram	*Gram*	%
0.0100	0.0468	21.37
0.0100	0.0474	21.10
0.0100	0.0472	21.19
0.0071	0.0334	21.26
0.0050	0.0236	21.19
0.0050	0.0234	21.37
0.0050	0.0236	21.19
0.0050	0.0235	21.28
	Av.	21.24
	Theoretical	21.22

Transfer an appropriate weight of sample, from 1 to 10 grams depending on the copper content and the accuracy desired, to a 400-ml., or larger, beaker. Add 25 ml. of nitric acid (sp. gr. 1.13) per gram of sample and heat until dissolution of the sample is complete and the oxides of nitrogen have been expelled. If more than 0.50 per cent of chromium is present the sample may be dissolved in hydrochloric acid and the iron subsequently oxidized by nitric acid. Add 7 grams of tartaric acid per gram of iron and 10 ml. of acetic acid, and adjust the volume to 200 to 300 ml. Make just alkaline to litmus with ammonium hydroxide and add 50 ml. of a 2 per cent aqueous solution of benzotriazole. Digest on a warm plate until the precipitate coagulates. Introduce paper pulp, stir, and filter through a Whatman No. 40, or equivalent, filter paper, washing the precipitate thoroughly with cold water. Transfer the paper and precipitate to a crucible and ignite at red heat, preferably in a muffle. Brush the ignited copper oxide into a 250-ml. beaker, add 10 ml. of nitric acid, and evaporate just to dryness on a hot plate. Add 20 ml. of water and, dropwise, sufficient 6 per cent sodium hydroxide solution to produce a permanent precipitate. Add 10 ml. of acetic acid and boil until the hydroxide dissolves. Cool to room temperature, add 6 ml. of 50 per cent potassium iodide solution, and titrate the liberated iodine with standard sodium thiosulfate solution, using starch as indicator.

Reagents. Nitric acid, specific gravity 1.13 and 1.42, glacial acetic acid, C. P. tartaric acid, and ammonium hydroxide, specific gravity 0.90.

Benzotriazole solution, 2.0 per cent by weight in water (may be purchased from the Eastman Kodak Company, Rochester, N. Y.).

Sodium hydroxide, 6 per cent by weight in water, potassium iodide, 50 per cent by weight in water, standard sodium thiosulfate solution, and starch indicator solution.

Application of Benzotriazole Separation of Copper to Analysis of Bureau of Standards Steels and Cast Irons. Table VI shows the results obtained on standard steels issued by the National Bureau of Standards, using the procedure described above.

Analysis of Copper Ores. The separation was used on copper ores. The copper contents of the ores were established by the electrolytic method which consisted of decomposing in nitric-sulfuric acid, diluting, filtering off the residue, and electrolyzing the filtrate using stationary electrodes. The benzotriazole-iodide method consisted of separating the copper as copper triazole and analyzing by the potassium iodide–thiosulfate method described for the iron and steel samples.

Table VII shows the results obtained on two copper ores by the two methods.

Discussion

The benzotriazole precipitation is specific for copper, and determinations may be made gravimetrically unless such ions as Ag^{+}, Ni^{++}, Fe^{++}, Cd^{++}, Zn^{++}, and Co^{++} are present. In the analysis of iron and steel interfering elements are likely to be present; so the separation is only preliminary and the final determination of copper is by a standard method. Because the potassium iodide–thiosulfate method is most widely used in industry this discussion relates largely to advantages and disadvantages of hydrogen sulfide (or hypo) versus benzotriazole precipitation of copper preparatory to the potassium iodide–thiosulfate determination. Other standard methods of determining copper besides the potassium iodide–thiosulfate can be employed.

Table VI. Analyses of Bureau of Standards Samples

Bureau of Standards Sample No.	Weight	Copper Present	Copper Found	Difference
	Grams	%	%	%
8e	10.0	0.008	0.008	0.000
9c	5.0	0.020	0.020	0.000
55a	5.0	0.046	0.045	−0.001
16c	5.0	0.060	0.062	+0.002
15b	3.0	0.145[a]	0.153	+0.008
34a	2.0	0.222	0.223	+0.001

[a] Ten laboratories reported on this sample. The results varied from 0.136 to 0.153 per cent of copper. J. I. Hofman of the Bureau of Standards reported 0.148, whereas the ferrous laboratory reported 0.153 per cent.

Table VII. Analysis of Copper Ores by Electrolysis and Benzotriazole

Weight	Copper Found	
	Electrolysis	Benzotriazole iodide
Gram	%	%
1.0	1.03	1.03
1.0	1.03	1.03
1.0	1.02	1.01
0.5	3.25	3.25
0.5	3.25	3.26
0.5	3.26	3.26

Advantages and Disadvantages

The benzotriazole separation in the determination of copper in cast irons and steels possesses the following advantages over the hydrogen sulfide–iodide–thiosulfate method:

It permits a more rapid dissolution of the sample than is possible in the other procedure, wherein a nonoxidizing acid attack is advisable.

It permits a more complete dissolution of the sample. This is most helpful in the analysis of certain cast irons which are not sufficiently decomposed by nonoxidizing acids to yield a sulfide precipitate free from iron.

It affords a clean separation from Mo^{++++++}, Se^{++++}, Te^{++++}, Sb^{+++++}, and As^{+++++}, which may interfere in the iodide titration.

The copper-triazole precipitate may be ignited more rapidly than the copper sulfide precipitate with less danger of fusion.

The disadvantages attending the benzotriazole separation are:

The reagent is expensive, 100 grams costing approximately $25, and about 1 gram is used per determination.

The conditions for precipitation are comparatively narrow (pH 7.0 to 8.5), whereas extreme latitude is permitted in the sulfide precipitation.

The separation by benzotriazole is not practical for materials containing large amounts of nickel, cobalt, silver, cadmium, and zinc because these metals also precipitate, and a large bulk of precipitate difficult to filter and ignite results.

Acknowledgments

The writer wishes to acknowledge the permission of the Battelle Memorial Institute, which financed the investigation, to publish the results, and his indebtedness to John D. Sullivan, James F. Shea, and Arnold E. Pavlish for valuable suggestions and assistance in the preparation of this paper.

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A Vacuum Tube Time-Delay Relay

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A NECESSARY auxiliary for many laboratory appliances and industrial devices is a time-delay relay or switch. The control of compressors, refrigerators, and other motor-driven devices which are likely to be injured by the "chattering" or the too frequent operation of a sensitive relay, is greatly facilitated by the use of a time-delay switch. The automatic application of plate voltage to thyratron rectifiers or x-ray tubes at a given interval after the filament current has been turned on is a convenience afforded only by an automatic time-delay relay. Although the device presented here was designed primarily for use in conjunction with a sensitive vacuum tube relay (*3*) for the operation of a thermostatically controlled refrigerator, many other uses may be suggested.

Very short time-delay action, from a small fraction of a second to a few seconds, may be obtained from a vacuum tube relay by placing a high-capacity condenser across the terminals of the magnetic relay (*1*, *3*). Although this procedure may limit chattering, the time-delay feature is a function of the rate of charge and discharge of the condenser and consequently its flexibility is limited to the comparatively narrow range afforded by condensers of practical size. Several commercial types of relays are available in which the time-delay action is obtained by a clockwork mechanism which trips a switch. Although a wide range of time-delay intervals is made available by this type of mechanism, the complexity of the clockwork trip unit makes its cost prohibitive in many instances. Other time-delay relays based upon the principle of the thermal inertia of an electrically heated bimetallic element are commercially available. These units offer difficulties of construction for the average laboratory technician.

Le Van (*2*) has utilized the cathode heating time of a vacuum tube as the basis of a time-delay switch. The unit described below likewise obtains time-delay action from the heating time necessary to allow electrons to flow from the cathode to plate of a vacuum tube rectifier. The circuit provides a simple, inexpensive, reliable, and easily constructed alternative time-delay device, which can be operated by a bimetallic thermoregulator of the type whose contacts open when the temperature setting is exceeded.

A schematic diagram of a single-tube relay unit is shown in Figure 1 *A*. When contact is made across contacts *XX* by means of a switch or relay, heater current begins to flow in the vacuum tube rectifier, *T*-1. When the cathodes of this tube reach a temperature sufficient to allow electrons to flow to the plates, the relay magnet becomes energized. The relay closes when *T*-1 has reached a temperature sufficiently high to allow the passage of the minimum amount of current which will offset the relay spring tension, and remains closed as long as the cathodes of *T*-1 exceed this minimum temperature.

If the circuit across the contacts *XX* is broken, the relay does not immediately release. Instead, the heater of *T*-1 slowly cools, causing a gradual decrease in the passage of current from the cathodes to the plates and through the relay coil. When the flow of current is no longer sufficient to offset the relay spring tension, the load circuit is opened.

Large variations in time interval may be obtained by increasing or decreasing the number of rectifier tubes used in cascade. Thus, one or more rectifier tubes connected as shown in Figure 1 *B* may be inserted in the relay unit shown in *A* at the lettered points.

When contact is made across contacts *XX* in a two-tube circuit of the type described above, heater current begins to flow in rectifier *T*-1. When the cathodes of this tube reach a temperature sufficient to allow electrons to flow, the heater of rectifier *T*-2 starts to heat, since the direct current load of the first rectifier is the heater of rectifier of *T*-2. As the second rectifier begins to pass current, the relay magnet becomes energized, since it is now directly connected across the output of the second rectifier.

Starting time delays up to 3.5 minutes have been obtained by using a series of five tubes connected in such a fashion that the output of each tube is used to supply the heater of the following rectifier. Changes in time interval may be accomplished by by-passing one or several tubes in the cascade. Rapid changeover may be facilitated by simply removing one or more tubes and replacing them with dummy plugs, certain terminals of which have been connected as shown in Figure 2.

Minor changes in the magnitude of the time-delay interval may be obtained by the adjustment of the spring tension on the relay contacts. An increase of spring tension will require a greater relay-actuating current, which will necessitate a longer heating period for the final rectifier tube. Although an increase in spring tension will cause an increase in the starting time interval, a corresponding decrease in the cooling or open contact delay will be produced. Since maximum reliability of operation is obtained at one definite adjustment of spring tension, only slight changes in time delay action may be made by this method.

A third method of producing variations in the time-delay interval is realized by changing the capacity of condenser *C*-1. Although the main purpose of this condenser is to prevent chattering of the relay when an alternating current power supply is used, slight time-delay variations may be

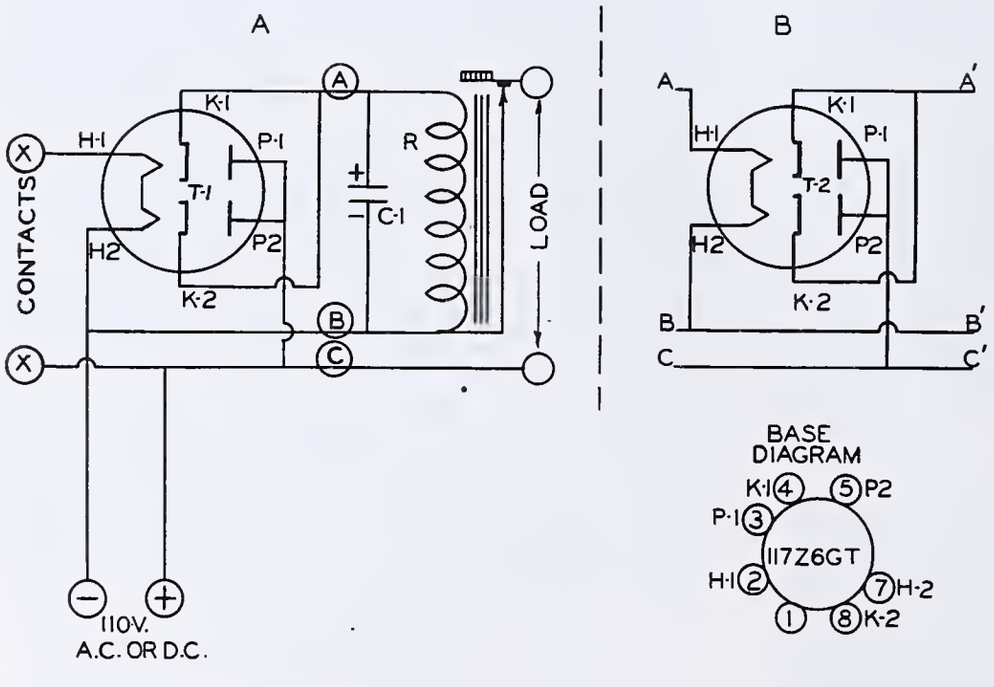

FIGURE 1. SCHEMATIC DIAGRAM OF TIME-DELAY RELAY

Relay operated by direct current, 115 to 135 volts to energize at 60 milliamperes or less
C-1. 8-mfd. electrolytic condenser, 250-volt working voltage
T-1 *T*-2. 117Z6GT vacuum tube, National Union Co.

gained by changing its capacity within practical limits (1 to 16 microfarads). If a high-capacity C-1 is used when 115-volt alternating current is applied, the direct current potential across the relay coil may reach as high as 160 volts. Danger of injury to the relay coil may result unless a current-limiting resistor is placed in series with the coil.

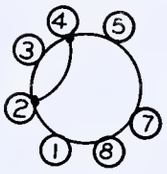

FIGURE 2

The time-delay relay described above offers a number of advantages over many of the commercially available devices, despite its simplicity of construction and low cost. Since standard radio replacement parts were used throughout the construction, the total cost of parts for the two-tube unit did not exceed $6.50. The use of rectifier tubes which require 117 volts for heater operation eliminates the necessity for filament current-limiting resistors or transformers and consequently permits the use of several tubes in cascade. Furthermore, the design permits the operation of the relay unit from either alternating or direct current power supplies. When a direct current power supply is used, proper polarity must be observed, as shown in Figure 1.

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Calibration of Existing Gum-Stability Test Bombs

In Terms of the New A. S. T. M. Bomb

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A method is presented whereby induction period bombs of non-A. S. T. M. type may be so calibrated as to yield "A. S. T. M. induction periods". This permits comparable results without the large expense of replacing useful equipment now in existence.

IT IS now well known that the deterioration of gasoline in storage, leading to the formation of gum, is a manifestation of atmospheric oxidation. It is accordingly reasonable that some form of an accelerated oxidation test employing air or oxygen at elevated temperatures will be required as a measure of stability. In 1930, Hunn, Fischer, and Blackwood (*3*) introduced a bomb test for this purpose, employing a temperature of 100° C. and an oxygen pressure of 7 kg. per sq. cm. (100 pounds per sq. inch) gage. Since that time, the bomb test has been rather widely adopted and used in one form or another—i. e., the length of the induction period or the gum formed after a given oxidation time. It has been rather difficult, however, to compare the bomb results obtained in different laboratories because of important differences in both the equipment and the testing procedure employed (*5*). Recently the American Society for Testing Materials has met this need by standardizing the bomb equipment and the testing procedure.

In this laboratory, there are already available twenty bombs of a type different from that specified by the A. S. T. M., and it would be advantageous to be able to calibrate them so that they would yield induction periods equal to those obtained by the A. S. T. M. bomb and procedure. Such a calibration would obviate the necessity for replacing useful and expensive equipment in order to obtain results comparable to those from other laboratories. This situation undoubtedly holds also for many other laboratories. An investigation was accordingly undertaken with the above objective in mind. The A. S. T. M. bomb would, of course, be used for referee purposes.

Equipment

The A. S. T. M. bomb setup was purchased complete from one of the equipment manufacturers, and unless otherwise noted was operated according to A. S. T. M. specifications.

The bombs already available in this laboratory are of the Universal Oil Products type (*2*). They were operated "in place" starting with the bath at room temperature, and they used a 100-ml. gasoline sample contained in a 240-ml. (8-ounce) oil sample bottle. The induction periods obtained with the U. O. P. bombs were selected according to A. S. T. M. specifications.

Temperature Correction

The variation of the induction period with the temperature can be expressed by the equation (*1*):

$$\log \text{induction period} = A + B/T$$

where A and B are constants depending on the particular gasoline, and T is the absolute temperature. The value of the slope, B, of this equation, when the temperature is expressed in degrees Kelvin, falls between the limits of about 5000 to 6500, averaging close to 5500, on the basis of a very large number of tests made in this laboratory with different gasolines. Over a fairly narrow temperature range (± about 2° C.), a simplified correction factor can be introduced which deviates only slightly from the rigorous equation given above. Considering now induction period determinations made in the range of 98° to 102° C., we can correct these to 100° C. by use of the simple factor:

$$F = 1 + 0.1\ \Delta T \qquad (1)$$

Here, ΔT is the difference between the reference temperature, 100° C., and the operating temperature (in the range 98° to 102° C.), and is always positive, so that F is always larger than 1. The observed induction period is multiplied by this factor, F, when the operating temperature is about 100° C. and divided by it when below 100° C.

In Table I are given induction periods determined at 98° and 102°, corrected to 100° by the exact equation and by the simplified factor, choosing materials having an extreme difference in temperature susceptibility characteristics (B = 5000

and 6500). The deviations shown in the last column become smaller as the operating temperature more nearly approaches 100° C.

Calibration of U. O. P. Bombs to Give A. S. T. M. Induction Periods

Let us assume that induction periods are determined in the A. S. T. M. bomb at a bath temperature of T_1, and in the U. O. P. bomb at a bath temperature of T_2. These will then be designated as $IP_{\text{A. S. T. M.}, T_1}$ and $IP_{\text{U. O. P.}, T_2}$. The temperature correction factors (see Equation 1) corresponding to the A. S. T. M. and U. O. P. induction periods (at bath temperatures T_1 and T_2) will be denoted by F_1 and F_2, respectively. As a matter of simplicity, it will be assumed in this case that T_1 and T_2 are both above 100° C., whereupon

$$(IP_{\text{A. S. T. M.}, T_1})\, F_1 = IP_{100^\circ \text{ C.}} \qquad (2)$$

$$(IP_{\text{U. O. P.}, T_2} - C)\, F_2 = IP_{100^\circ \text{ C.}} \qquad (3)$$

Equation 2 is self-evident; in Equation 3 we have first subtracted a correction factor, C, from the observed induction period before applying the temperature correction factor, F_2. C corrects for the heating-up lag of the gasoline in the U. O. P. bomb, and also for any difference in the equilibrium gasoline temperature in the A. S. T. M. and U. O. P. bombs (when the respective bath temperatures are the same). Combining Equations 2 and 3, we obtain

$$C = (IP_{\text{U. O. P.}, T_2}) - \frac{F_1}{F_2}(IP_{\text{A. S. T. M.}, T_1}) \qquad (4)$$

(If T_1 and T_2 were both below 100° C., the F factor of Equation 4 would be F_2/F_1. If T_1 were above 100° C. and T_2 below 100° C., the F factor would be F_1F_2.)

Now we know from previous work that the equilibrium gasoline temperature in the U. O. P. bomb is some 0.6° C. below the observed bath temperature, but we would guess that the equilibrium gasoline temperature in the A. S. T. M. bomb is practically the same as its bath temperature. [Gasoline temperatures have been obtained during the heating-up period of the U. O. P. bombs by means of thermocouples fitted through a special bomb top. The time correction to be applied to an observed U. O. P. induction period in order to obtain an isothermal induction period is 30 minutes for this particular bath setup, as computed from the heating curves (*1*, *4*).] Accordingly, the value of C will depend on the length of the induction period. In determining C for any type of bomb, it is not necessary to know the actual temperature lag or the equilibrium temperature level reached by the gasoline in the bomb. We merely run a number of induction periods in both the A. S. T. M. and unknown bombs and calculate C by means of Equation 4. C is then plotted against the length of the induction period observed for the unknown bomb—i. e., at a bath temperature of T_2—and a best straight line is drawn through the points, corresponding to the equation

$$C = m\,(IP_{\text{obs.}, T_2}) + b \qquad (5)$$

If the value of m equals 0 (perfectly horizontal line), then the equilibrium gasoline temperature is the same in the unknown bomb as in the A. S. T. M. bomb, for the same bath temperature. If the value of b equals 0, then the heating-up lag of the gasoline in the unknown bomb is the same as that in the A. S. T. M. bomb.

Once we have established the characteristics of C, we may employ the unknown bomb and obtain induction periods equivalent to those in the A. S. T. M. bomb at 100° C. by means of Equation 3. Equation 3 as written assumes the bath temperature, T_2, of the unknown bomb (in this case, U. O. P.) to be above 100° C. If T_2 were below 100° C., Equation 3 would read

$$(IP_{\text{obs.}, T_2} - C)/F_2 = IP_{100^\circ \text{ C.}}$$

Table I. Induction Periods Calculated by Simplified Factor Method

Operating Temperature, T ° C.	B Values Assumed	Induction Periods: Observed values Min.	Corrected to 100° C.: Exact equation Min.	Corrected to 100° C.: Simplified factor Min.	Error by Use of Simplified Factor Min.
102	5000	204	240	245	+5
102	6500	194	240	233	−7
98	5000	283	240	236	−4
98	6500	298	240	248	+8

Table II. Correlation between A. S. T. M. and U. O. P. Induction Periods

Gasoline Item No.	Bath Temperature A. S. T. M. (T_1) ° C.	Bath Temperature U. O. P. (T_2) ° C.	Observed Induction Period A. S. T. M. Min.	Observed Induction Period U. O. P. Min.	C for U. O. P. Bomb: Calcd. by Equation 4 Min.	C for U. O. P. Bomb: Smoothed value from Figure 1 Min.	Induction Periods, Corrected to 100° C. A. S. T. M. Min.	Induction Periods, Corrected to 100° C. U. O. P.[a] Min.	Deviation of Corrected U. O. P. from Corrected A. S. T. M. Induction Period Min.	Deviation of Corrected U. O. P. from Corrected A. S. T. M. Induction Period %
1	101.1	101.1	20	40	20	23	22	19	− 3	−14
2	101.0	101.0	20	45	25	23	22	24	+ 2	+ 9
3	100.9	101.0	20	45	25	23	22	24	+ 2	+ 9
4	101.0	101.0	30	55	25	24	33	34	+ 1	+ 3
5	101.0	101.0	45	70	25	25	50	50	0	0
6	101.0	101.0	95	120	25	28	105	101	− 4	− 4
7	101.0	101.1	150	175	26	32	165	159	− 6	− 4
8	101.1	101.2	160	175	16	32	178	160	−18	−10
9	100.8	100.8	195	215	20	35	211	194	−17	− 8
10	101.0	101.0	205	240	35	37	226	223	− 3	− 1
11	101.0	101.1	220	265	47	39	242	251	+ 9	+ 4
12	101.0	101.1	255	295	42	41	281	282	+ 1	0
13	100.8	101.1	270	295	32	41	292	282	−10	− 3
14	101.0	100.9	255	300	43	41	281	282	+ 1	0
15	100.8	100.8	250	305	55	41	270	285	+15	+ 6
16	100.8	101.1	290	320	38	42	313	309	− 4	− 1
17	100.9	101.0	285	340	58	44	311	326	+15	+ 5
18	101.1	101.2	295	345	53	44	327	337	+10	+ 3
19	100.6	100.8	350	410	66	49	371	390	+19	+ 5
20	101.1	101.2	395	435	44	50	438	431	− 7	− 2
21	100.9	101.0	400	470	74	53	436	459	+23	+ 5
22	101.0	101.0	430	495	65	55	473	484	+11	+ 2
23	101.0	101.0	460	510	50	56	506	499	− 7	− 1
24	101.0	101.1	470	515	49	56	517	509	− 8	− 2
25	100.8	100.9	465	530	69	57	502	516	+14	+ 3
26	101.0	101.1	530	635	110	64	583	634	+51	+ 9
27	101.0	101.1	595	645	55	65	655	644	−11	− 2
28	101.0	101.0	580	655	75	66	638	648	+10	+ 2
29	101.0	101.1	670	725	61	71	737	726	−11	− 1
30	100.9	101.0	870	920	58	84	948	920	−28	− 3

[a] Using smoothed value of C taken from Figure 1 (column 7).

In order to test the above relations, induction periods were determined for 30 different gasoline samples in both the A. S. T. M. and U. O. P. bombs. A. S. T. M. induction periods were obtained with 50 ml. of gasoline instead of 100 ml., but all other A. S. T. M. specifications were followed in carrying through the procedure. U. O. P. induction periods were obtained with 100 ml. of gasoline in a 240-ml. (8-ounce) oil sample bottle, and the U. O. P. bombs were operated "in place"; the induction periods were selected according to A. S. T. M. specifications. To obtain gasolines differing in type and in the length of the induction period, a number of base stock gasolines were treated to various extents with caustic solution to reduce the induction period; in order to lengthen the induction period, several different inhibitors were added to the variously treated base stock gasolines.

The data and calculations are presented in Table II. Column 6 gives the values of C calculated by means of Equation 4. These are plotted against the observed U. O. P. induction period ($IP_{U.O.P.,T_2}$) in Figure 1. The line drawn through the points corresponds to the equation (see Equation 5)

$$C = 0.07\,(IP_{U.O.P.,T_2}) + 20 \qquad (6)$$

This would indicate a temperature lag of about 20 minutes and an equilibrium gasoline temperature about 0.7° C. below that of the bath temperature, in comparison with the A. S. T. M. bomb. These figures are in very good agreement with previous values obtained directly—i. e., 25 minutes and 0.6° C. (A temperature lag of about 5 minutes is assumed for the A. S. T. M. bomb. The difference in temperature lag for the two bombs is thus 25 minutes.) A small change in the position of the line of Figure 1 may appreciably change the indicated lag and temperature level figures noted above, but will have a negligible effect on the final corrected induction period. Indeed, in placing the line of Figure 1, a considerable amount of latitude may be allowed, since a displacement corresponding to a 5-minute change in C at any particular point will raise or lower the induction period by only this amount.

Column 7 of Table II lists the "smoothed" values of C taken from Figure 1 (actually calculated by means of Equation 6) and these were used to calculate U. O. P. induction

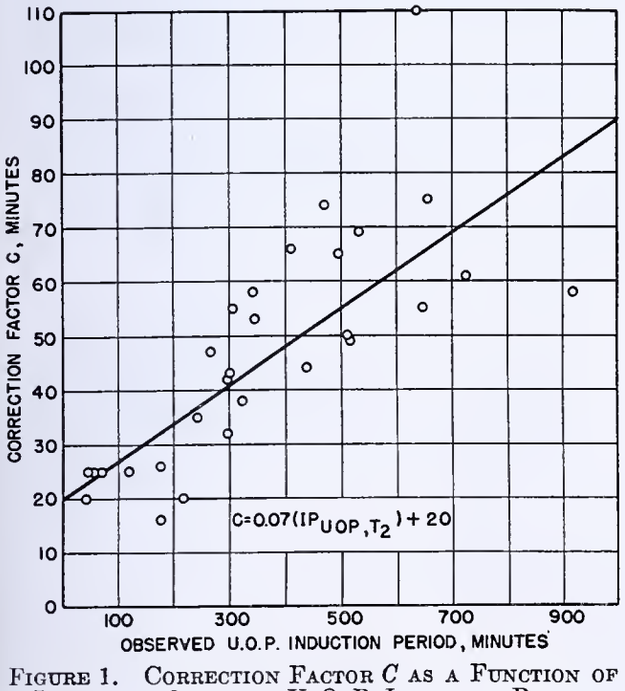

Figure 1. Correction Factor C as a Function of Length of Observed U. O. P. Induction Period

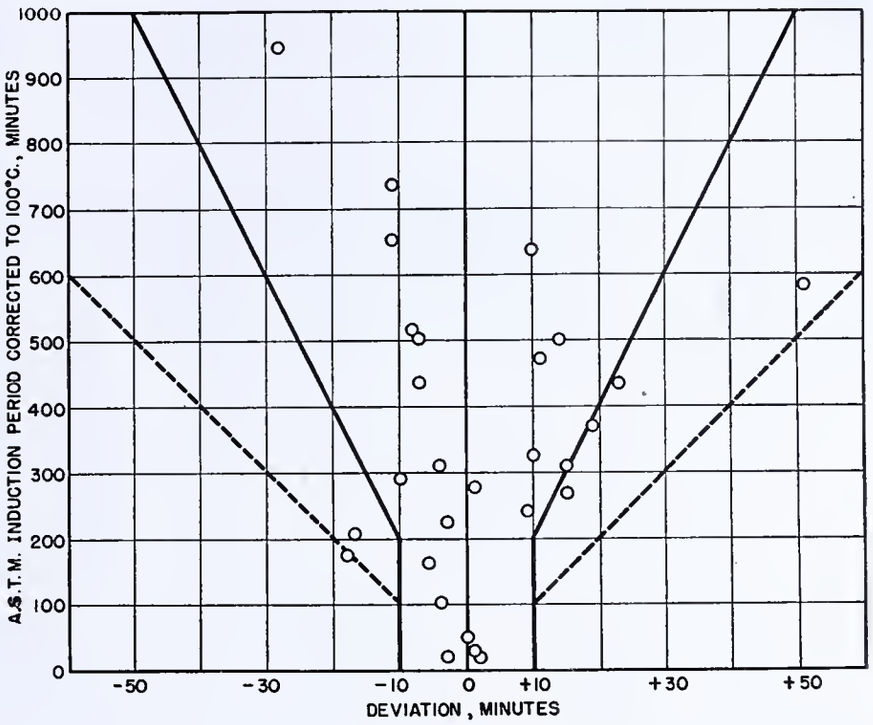

Figure 2. Deviation of Corrected U. O. P. Induction Period from Corrected A. S. T. M. Induction Period

Solid "funnel" indicates 5 per cent deviation, broken line indicates 10 per cent deviation

periods corrected to 100° C. (by means of Equation 3) as given in column 9 of Table II. The A. S. T. M. induction periods were corrected to 100° C. in the usual way by means of Equation 2 and these are listed in column 8. The deviations of the completely corrected U. O. P. induction periods from the corrected A. S. T. M. induction periods are shown in columns 10 and 11, and are plotted in Figure 2. The solid lines forming the "funnel" of Figure 2 indicate a deviation of 10 minutes up to an induction period of 200 minutes, and 5 per cent thereafter. The dotted lines forming the larger funnel indicate a 10 per cent deviation beyond 100 minutes. The points fall well within the 5 per cent funnel on the whole. The actual distribution of points is as follows:

Deviation of Corrected U. O. P. Induction Period from Corrected A. S. T. M. Induction Period			Number of Points
I.	0 to 10 minutes		18
II.	Greater than 10 minutes		
	a. 0 to 5% deviation	8	
	b. 6 to 10% deviation	4	12
	Total		30

Of the 4 points that deviated by more than 10 minutes and 5 per cent (II, *b*, above) from the corrected A. S. T. M. induction period, a change of 2 minutes in the induction period would bring 1 of these points within the 5 per cent limit and a change of 10 minutes would bring 2 more within this limit.

Since the A. S. T. M. method allows a 15-minute deviation of duplicate A. S. T. M. induction periods by the same operator, or one of 20 per cent of the smaller value for different laboratories, the above results indicate that a calibration of existing bombs, in the manner described herein, is capable of yielding corrected induction periods equal to those obtained with the A. S. T. M. bomb, well within the limit of error of the A. S. T. M. method.

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Inexpensive Laboratory Circulating Pump

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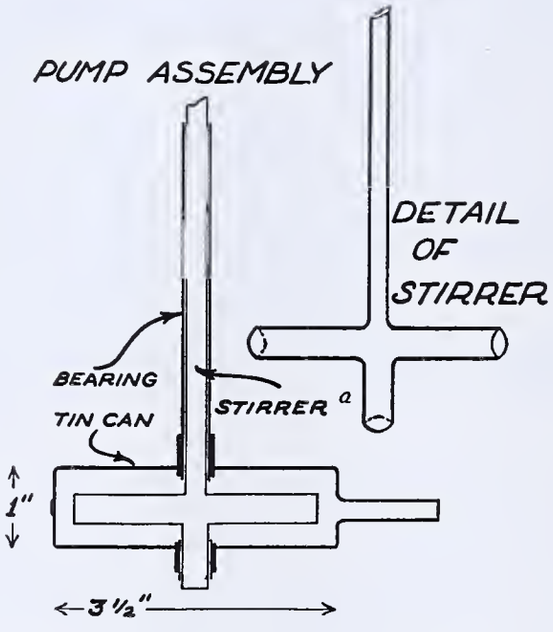
IN MOST laboratories there is occasional need for a means of circulating water or other liquids at definite temperature through a piece of equipment such as a refractometer, polarimeter, or condenser. This paper describes a simple pump devised for that purpose which can be constructed in a short time, at very little expense, and with a minimum of glass-blowing and shop technique.



The essential parts of the pump are a long-shanked, glass, "centrifugal stirrer" (*1*) and a flat circular tin can with a tightly fitting cover. A large-sized shoe-polish can or ointment can is ideal.

A centrifugal stirrer by its rotation sucks liquid up through the center and throws it out through the hollow arms. For use in the pump, the stirrer is made in slightly modified form as shown in the diagram. Nine-millimeter or larger Pyrex tubing is suitable for the lower part of the stirrer, while the shank may be of smaller diameter. The length of the stirring arms will depend on the diameter of the can used and to a certain extent also on the purpose for which the pump is made. In general, short arms with considerable distance between their ends and the casing produce maximum flow and low lift, while long arms with ends close to the casing result in a higher lift and somewhat diminished flow. In this laboratory most of the pumps made have allowed about 0.63 cm. (0.25 inch) of space at the ends of the arms when the pump is assembled. Tube *a* of the stirrer need be only long enough to protrude 1.25 cm. (0.5 inch) through the bottom of the can.

Holes (1.25 cm., 0.5 inch) are drilled through the centers of the top and bottom of the can and a 0.63-cm. (0.25-inch) brass or copper outlet tube is soldered on at the side. Bearings for the stirrer are made from the proper size of glass tubing and are attached to the can by small sections of rubber tubing slipped over the glass and into the holes in the can. The parts of the stirrer which run in the bearings are greased before assembling. The long shank and bearing of the stirrer prevent pressure inside the pump from forcing liquid up through the bearing rather than out the side tube.

The pump is operated by any kind of variable-speed motor, its rotation being free enough so that even a small compressed air turbine or wind motor (*2*) supplies sufficient power. It may be completely immersed in the liquid to be circulated or may have only the protruding lower tube immersed. In the latter case, it may be necessary to prime the pump before it will work.

Several pumps of this kind have given surprisingly good performance in this laboratory. Tests made on one pump of the dimensions shown in the diagram, running at only a moderate speed, gave a pumping rate of 1300 ml. per minute through a horizontal line and 650 ml. per minute when water was lifted 60 cm. (2 feet). The maximum lift at this speed was about 90 cm. (3 feet), though, of course, higher speeds would increase both lift and pumping rate.

Changes in design, such as the use of better bearings, or the attachment of the discharge tube tangentially instead of radially, would undoubtedly increase efficiency. However, the desire has been to keep the device simple and easy to make, and the cost low. A coat of water-resistant varnish would make the can last longer in contact with water.

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A Convenient Distilling Column Head

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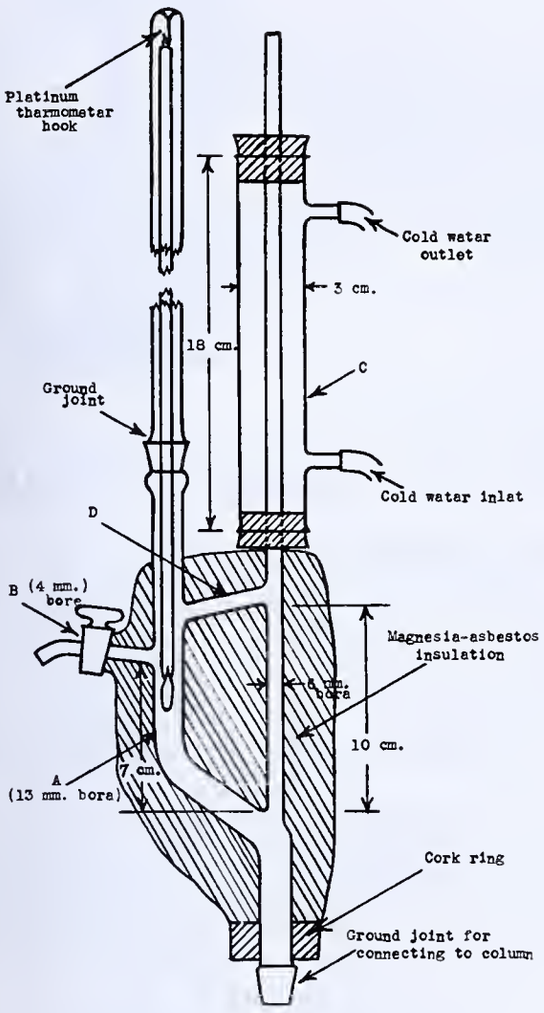
DURING the construction of a number of highly efficient fractionating columns needed in this laboratory for the preparation of spectroscopically pure samples of various organic compounds, the question of the type of column head best suited to the problem came to the fore. Most of the authorities consulted seemed to prefer the totally condensing head, because of the greater ease of control of product and reflux rates, the readiness with which the changing conditions at the head can be followed by temperature measurements, and the lack of necessity for the careful control of the condenser. Unfortunately, however, totally condensing heads are so constructed that the condensate formed when the column is first started is continuously diluted during the attainment of equilibrium conditions. Any impurity in the first condensate, therefore, is not completely washed out but is merely diluted and partly removed during the time required to establish equilibrium. For ordinary purposes the small quantity of impurity remaining would be insignificant; in ultraviolet absorption spectra, however, impurities present to the extent of 1 part in 10,000 often show strong absorption bands. For this reason it seemed advisable to design a new head which would, if possible, retain all the advantages of the totally condensing type, yet would permit the product to be removed in the vapor state and condensed in an external condenser.



The head which was finally designed is shown in the accompanying diagram. Essentially, it consists of a condenser, *C*, of sufficient capacity to condense all vapor reaching it; a thermometer well, *A*, which is offset so as to avoid any disturbance due to proximity of the condenser or to contact with the cooled reflux; and a large-bore stopcock, *B*, through which product, in vapor form, may be withdrawn through the thermometer well. All parts are so constructed and leveled that there are no indentations in which reflux can be retained; even the tube to the stopcock is short and slanted upward to permit condensate to return readily to the column. To allow vapor to pass freely through the thermometer well, a bridging tube, *D*, is sealed between the condenser tube and the thermometer well. The bridge, which is slanted downward in the direction of the well, catches a small fraction of the reflux and diverts it through the thermometer well to help prevent the accumulation of dead vapors in that space. In practice, the head is surrounded by a celluloid cylinder to lessen rapid temperature fluctuations due to air currents in the room. Heat leakage through this, however, causes a small condensation in the upper parts of the thermometer well and prevents the upper parts of the well from acting as a vapor pocket.

Tests made of the operating characteristics of this head have shown that it can be handled even by novices with a minimum of instruction. With the vaporization rate fixed, it is possible to control the reflux ratio accurately merely by controlling the product rate with the stopcock; and by checking the temperature with a thermometer having scale divisions of 0.1° C., it has been simple to follow the approach of the column to equilibrium under total reflux, to note with fair accuracy the time required for equilibrium to be attained, and to observe signs of flooding long before other signs than that given by the temperature change made their appearance.

Although the head has thus far been used only with columns of 1.2-cm. diameter and from 100- to 170-cm. height, packed with glass or Nichrome helices, its performance should be equally excellent with other laboratory columns.

Bromination of Phenols by Means of Bromide-Bromate Solution

SIR: A recent article (*4*) with the above title presented bromine titrations for a variety of substituted phenols, many of which show good agreement with theory. Others, however, including *o*- and *p*-cresols, four xylenols, and other alkyl phenols, were reported to be overbrominated under the conditions used by Sprung. He ascribed this discrepancy with theory to side-chain substitution.

These fractional titration values were considered reproducible, and if so, are satisfactory for some purposes. However, a method which gives accurate values agreeing with theory was described (*2*) several years ago and applied to phenols, aromatic amines, and nitro aromatics. We have used the method occasionally since then, and never had any trouble with overbromination of cresols.

Typical results giving the number of moles of bromine per mole of cresol are as follows:

o-Cresol	*m*-Cresol	*p*-Cresol
1.996	3.007	2.006
1.985	2.995	2.014
1.998	3.000	2.006
	3.007	

Also, 50 per cent *o*-cresol, 50 per cent *m*-cresol, 2.502 (theory 2.500); one third of each cresol, 2.337 (theory 2.333). Similar results (*1*) were obtained for the four xylenols which Sprung found were overbrominated.

The essential difference in procedure seems to be in the time of standing with excess bromine. The nuclear substitutions have velocity constants (*3*) of the order of at least 10^6, so that no delay is necessary after the appearance of free bromine, and the merest trace of excess is sufficient. Since the formation of bromine is a time reaction, however, an excess of bromide-bromate can be used; but by employing a lower concentration of acid (sulfuric or hydrochloric) the production of bromine can be controlled to any desired rate. As soon as a yellow color appears, bromination can be stopped by addition of potassium iodide. A sharp distinction between nuclear and side-chain substitution is thus possible. The iodine can be titrated immediately with sodium thiosulfate. To accelerate attainment of the end point more acid can be added before or during this titration.

Sprung used about eight moles of potassium bromide for one of potassium bromate. A much smaller proportion of bromide, a little over two moles, is sufficient, as explained in the original article (*2*). It may be that this lower concentration of salt facilitates accurate titration.

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Procedure for Semimicrodetermination of Sulfur in Organic Compounds

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A SATISFACTORY procedure for the semimicrodetermination of sulfur in organic compounds by use of the sodium peroxide bomb has hitherto been lacking, and no bomb equipment suitable for the purpose has been described. The feasibility of using the sodium peroxide macro- or microbomb for decomposition of semimicrosamples has doubtless been tested by many analysts. The few opinions available here with respect to the use of the microbomb in this way are contradictory. Some preliminary test analyses made with semimicrosamples and a macrobomb gave acceptable results when the charge consisted of about 0.03 gram of sample (taurine), 0.2 gram of potassium perchlorate, 0.2 gram of sucrose, and 8 grams of sodium peroxide. As this procedure appears to involve a deliberate imposition of unfavorable conditions for precipitation of barium sulfate, it has not been examined further.

This paper presents descriptions of a bomb assembly and an analytical procedure which permit the determination of sulfur in solid or liquid organic substances by decomposition of semimicrosamples in admixture with sodium peroxide, potassium perchlorate, and sucrose in a metal bomb, the sulfur being weighed as barium sulfate. The magnitudes of sample, combustion charge, and apparatus are intermediate between those of the familiar macroprocedure (*7*) and the microprocedure of Elek and Hill (*5*).

Parr Sodium Peroxide Semimicrobomb

Inquiry disclosed the fact that the Parr Instrument Company, Moline, Ill., had previously designed a bomb assembly with a cup of about 8-cc. capacity, and presumably suitable for semimicroanalysis. One of these outfits was secured and was found satisfactory.

The form of the apparatus, and some of the principal dimensions, are shown in Figure 1. The dimensions indicated are taken from blueprints provided by the Parr Instrument Company, from which the apparatus may be obtained. The cup is made of either 98 per cent nickel or 30 per cent nickel steel, and the cover of either 98 per cent nickel or nickel-plated brass. Neither stainless steel nor any other alloy of chromium is suitable for parts which are to be exposed to the ignited sodium peroxide mixture. The screw collars are made of chromium-plated bronze. Lead gaskets were used; rubber gaskets are available and would doubtless serve about as well. In either case, since the charge is ignited by applying a flame to the bomb, care must be taken not to overheat the gasket.

Gooch Crucibles for Sulfur Semimicrodeterminations

Porcelain Gooch crucibles of 10-cc. and 15-cc. capacities were used; the smaller size is preferred. Since in this method an attempt must be made to weigh the Gooch crucible to about 0.02 mg. it is important that the asbestos mat be properly formed, to avoid loss due to passage of fine asbestos fibers through the perforations, and especially that the prepared filter be brought

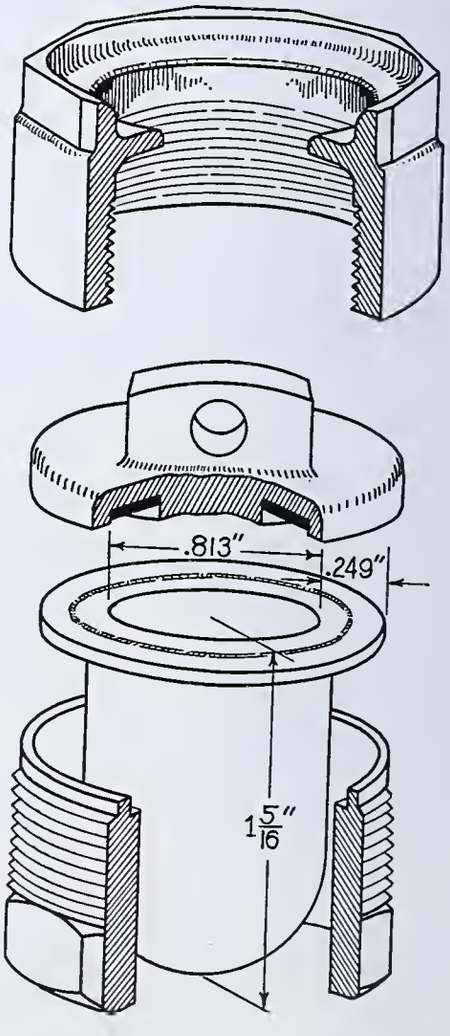

FIGURE 1. PARR SODIUM PEROXIDE SEMIMICROBOMB

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o constant weight under the conditions of its use in analysis. Constancy of weight should never be assumed. A solution, in which the volume, temperature, and concentrations of sodium chloride and hydrochloric acid approximate those of the liquid to be filtered in analysis, is passed through the filtering crucible, which is then washed, dried, ignited, and weighed by the procedures to be used in the analysis. This treatment should be repeated until the weight is constant within 0.05 mg.

The importance of these precautions is shown by the following data, obtained with four 10-cc. Gooch crucibles. Each crucible was prepared as usual, and was then treated with 150 cc. of a liquid whose temperature, acidity, and salt concentration approximated those of the analysis liquid. It was washed, dried, and weighed as described below.

Treatment with Conditioning Solution	Changes in Weight			
	Gooch I	Gooch II	Gooch III	Gooch IV
	Mg.	*Mg.*	*Mg.*	*Mg.*
1	Before first weighing			
2	+0.02	−0.28	−0.44	−0.02
3	....	−0.03	−0.01	....

The four crucibles did not behave uniformly; two attained constancy of weight after one treatment with the conditioning solution, while the other two required an additional treatment. The asbestos mat thus conditioned may be used for a considerable number of analyses. After five or more precipitates have accumulated, the greater part of the barium sulfate may be removed, dislodging not more than a superficial layer of asbestos. Enough fresh asbestos is introduced to restore the mat to its original thickness, and the crucible is brought to constant weight, an operation which should involve little or no trouble.

Conditions for Analysis

Preliminary experiments showed a mixture of 4 grams of sodium peroxide, 0.2 gram of potassium perchlorate, and sucrose and sample totaling 0.2 gram (0.02 to 0.05-gram sample) to effect satisfactory decompositions in the semimicrobomb. The conditions for precipitation and handling of the barium sulfate obtained from such charges are chosen with a view to controlling some of the errors which are unavoidable when the small precipitate of barium sulfate is formed in the presence of considerable sodium chloride, some barium chloride, hydrochloric acid, and sodium sulfate.

To avoid excessive contamination of barium sulfate by salts (*9*, *10*, *18*, *21*), precipitation is effected in a volume of about 150 cc. (a dilution greater than in the macromethod, *9*), and with a dilute precipitant added rapidly, as suggested by Hintz and Weber (*8*). This procedure has been found advantageous for macroprecipitations under like conditions (*8*, cf. *19*), though perhaps of reduced advantage in the present method because of the delay in the appearance and subsidence of the small precipitate of barium sulfate (*14*). The "reversed precipitation" appears to have some merits (*16*, *19*), but it has been reported to increase contamination by anions (*15*, *19*) and so was not tried. The acidity (1 cc. of concentrated hydrochloric acid in a volume of 150 cc.) is such that increase in the solubility of barium sulfate and occlusion of "free" sulfuric acid (sodium hydrogen sulfate, *2*) and of chlorides (*10*) are not favored. To minimize coprecipitation by adsorption on slowly growing crystals (*12*, *20*, *24*, *25*), and to render the precipitate more promptly filterable, granulation of the precipitate is hastened by addition of picric acid (*19*). Results which indicate the value of this procedure are given in Table IV.

No provision is made for avoiding interference of nitrate (*6*), which need be present only to the extent that it may be formed during decomposition of nitrogenous —e. g., nitro— compounds. No provision is made for avoiding interference by iron or chromium. The familiar devices for avoidance of contamination of barium sulfate precipitates by iron (*1*, *17*, *23*) are inconvenient or uncertain. Errors due to chromic ion are avoidable in part by addition of alkali acetate (*17*), but this increases the concentration of salts present. Chromate ion may cause serious contamination (*4*) because of coprecipitation of mixed crystals of isomorphous barium sulfate and barium chromate (*3*, *11*, *13*). It is therefore advisable to use a bomb which yields little or no iron or chromium, and to replace a cup or cover found to yield considerable of either. The nickel bomb and nickel-plated cover specified above are satisfactory; stainless steel and illium parts are not satisfactory.

Procedure

Transfer to the bomb cup 0.2 gram of powdered potassium perchlorate. Introduce the sample (finely powdered if a solid; for manipulation and analysis of liquids see below), which should be sufficient to yield from 20 to 100 mg. of barium sulfate, and should be weighed to 0.02 mg. Add sucrose in such amount that the sum of sample plus sucrose is 0.2 gram. Weigh out rapidly 4 grams (±0.1 gram) of granular sodium peroxide (the grade low in sulfur) and transfer to the cup. A satisfactory procedure, especially in humid weather, is to support the bomb cup on the left-hand pan of a trip scale, place a small beaker on the right-hand pan, and introduce water to counterpoise the bomb cup, into which the sodium peroxide is weighed directly and rapidly. A measuring cup of proper capacity would be a convenience.

Make certain that the gasket is in good condition, and then adjust the cover in place and secure it firmly by tightening the screw collars. To mix the charge shake the apparatus vigorously, and finally tap the cup against the table top to settle the charge. Support the apparatus in a vertical position by means of a clamp and ring stand, at such height that the cup can be heated by the flame of a blast lamp seated on the table top. It is advisable to wear goggles, and to place an iron plate or other protective device in front of or around the bomb while the charge is ignited. The explosion hazard which attends the use of this apparatus cannot yet be estimated. Sodium peroxide macrobombs sometimes explode with dangerous violence, but the factor of safety should be considerably greater with the smaller bomb because of its relatively heavier construction and the reduced charge which it contains. The first semimicrocup used developed a perforation in the bottom after about a hundred decompositions, and this presumably followed a gradual weakening of the wall which might have led to explosive rupture.

Adjust the blast lamp flame so that it is 7.5 cm. in length and 3 to 4 mm. in diameter. First bring the tip of the flame to a point about 1 cm. below the bomb for about 15 seconds, and then raise the flame so that it touches the bottom of the bomb. This gradual heating appears to prevent the projection of a portion of the charge against the inner surface of the cover, to which some of it may adhere and thus escape combustion. Heat the bomb in this way for 30 seconds; a heating period much longer than this may melt the gasket.

Cool the apparatus, finally, in tap water, and remove the cover. Wash the lower surface of the cover with hot distilled water, collecting the washings in a 250-cc. beaker. Wash the bomb externally (discard these washings) and then, with the aid of a thick glass rod, place the cup on its side in the beaker. Add water to a volume of about 50 cc., cover the beaker, and allow the contents of the cup to dissolve, if necessary warming gently at first to start the action. Using the glass rod lift out the bomb cup and wash it externally. Then grasp it between the fingers and wash the interior, collecting the washings with the main extract. Cover the beaker and allow the liquid to cool somewhat. Insert the glass rod obliquely, so that it projects through the lip of the beaker, push the watch glass slightly away from the rod, and then cautiously pour 8 cc. of concentrated hydrochloric acid along the sloping rod, so that the acid enters the liquid at a point well under the cover. This procedure minimizes loss due to spraying. Test a drop of the liquid on Congo red paper, and if necessary add more acid until the liquid is just acid in reaction.

To the solution add 1 cc. of concentrated hydrochloric acid in excess, and warm on the hot plate to expel most of the carbon dioxide. Filter the liquid through paper, receiving the filtrate in a 250-cc. beaker, and wash the first beaker, the watch glass, and finally the filter, with hot water. To the filtered solution add 15 cc. of a saturated aqueous solution of picric acid (about 1 per cent), and dilute the liquid to a volume of about 140 cc. Cover the beaker and heat the solution to boiling. Meanwhile measure out 7 to 10 cc. of 0.1 *N* barium chloride solution, heat to boiling, and then add it rapidly, with stirring, to the hot sulfate solution. Digest the liquid at or near 100° C. for an hour or more, when the precipitate may be removed by filtration. Alternatively allow the analysis to stand until the following day before filtering; in this case the picric acid is dispensable.

Decant the liquid through a weighed Gooch crucible (10-cc.),

Table I. Analysis of Potassium Sulfate

K_2SO_4	Sulfur	$BaSO_4$	Sulfur Found	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
100.31	18.45(5)	133.90	18.39(1)	−0.06
100.31	18.45(5)	134.06	18.41(3)	−0.04
100.31	18.45(5)	134.10	18.41(9)	−0.04

wash the precipitate several times with hot water by decantation, and then transfer it to the filtering crucible with the aid of a fine stream of hot water. Dislodge any barium sulfate adhering to the beaker and stirring rod by thorough manipulation with a small policeman and transfer to the filter. Wash the precipitate in the Gooch crucible five times with hot water. Dry the crucible in an oven at 120° for 30 minutes, and then transfer to a large porcelain crucible supported on a triangle above a Bunsen burner. Cover the larger crucible with its lid, and heat for 30 minutes with the full flame of the burner. Allow the Gooch crucible to cool somewhat, transfer it to a desiccator, and when it has cooled to room temperature set it in the balance case. After 15 minutes weigh the crucible to 0.02 mg.

The procedure for precipitation and eventual weighing of barium sulfate, as described in the last two paragraphs, was tested in preliminary trials. Aliquot portions of a standard solution of potassium sulfate (recrystallized from hot water, dried at 120°, ground in a mortar, redried at 120°, and kept in a desiccator over calcium chloride) were analyzed, with addition of 5 grams of sodium chloride to simulate the conditions encountered in the analysis of organic compounds. The results of the final trials appear in Table I.

Blank Analysis. Blank analyses were conducted by the procedure described, using 0.2 gram of sucrose and omitting the sample. The blanks obtained in the experimental trials yielded precipitates not visible, but filtration showed presence of insoluble material. It seems advisable to conduct blanks in triplicate, and to use the averaged result, as individual results may show variations. Some results obtained by the entire procedure described above, applied to fifteen organic compounds, are collected in Table II.

Procedure for Analysis of Liquids. Liquid substances which are not volatile or sensitive to atmospheric moisture, and which are not attacked by sodium peroxide upon contact, may be weighed from a Lunge-Rey pipet or other similar device, directly into the bomb cup. Liquids which cannot safely be handled in this way must be weighed and introduced into the bomb in sealed thin-walled glass ampoules, which can be broken during the mixing of the charge after the bomb is closed. The decomposition in the bomb then effects a silica fusion of the thinner parts of the glass bulb, only the thicker glass of the stem ordinarily surviving the action. [Rapid silica fusions of minerals might be possible if samples were ground impalpably fine. A specimen of orthoclase, submitted to decomposition in a macrobomb, was rendered water-soluble except for a small residue of the less finely ground material (*22*).]

Acidification of the aqueous extract leads to separation of gelatinous silicic acid, which must be removed before the barium sulfate is precipitated. The interference of silicic acid following decomposition of samples contained in glass ampoules is not mentioned in familiar descriptions of the sodium peroxide bomb procedure. This consequence of the use of a glass container for an organic sample may affect analyses for any element determinable by use of the sodium peroxide bomb, especially if a gravimetric method is to be employed. Some comments on the obstruction offered by silicic acid in the determination of halogen in liquid substances will appear in a later paper.

The removal of silicic acid is effected by the dehydration procedure familiar in mineral analysis.

After extraction of the ignited charge acidify the liquid, evaporate to dryness on a steam bath, and moisten the dry residue with 5 cc. of concentrated hydrochloric acid. Again evaporate to dryness, and heat the residue for 1 to 2 hours on the steam bath. Extract soluble material in about 75 cc. of hot water, pass the extract through a paper filter, transfer the silica residue to the filter, and wash five times with hot water. Add 1 cc. of concentrated hydrochloric acid and 15 cc. of saturated picric acid solution. Dilute the liquid to 140 cc., and then proceed as described above.

Some results which indicate the usefulness of this procedure are given in Table III.

Effect of Picric Acid on Semimicroprecipitation of Barium Sulfate

Some trials of the procedure described were made in the presence and absence of picric acid to test its effectiveness

Table II. Semimicrodetermination of Sulfur in Organic Compounds

(By use of sodium peroxide bomb)

Compound	Sample	$BaSO_4$	Sulfur		
			Calcd.	Found	Difference
	Mg.	*Mg.*	%	%	%
Sulfanilic acid	41.61	55.84	18.52	18.43	−0.09
	28.55	38.31		18.43	−0.09
p-Toluenesulfonamide	32.19	44.48	18.71	18.98	+0.27
	38.30	53.20		19.08	+0.37
N - *p* - tolylbenzenesulfonamide (m. p. 122° corrected)	49.05	46.37	12.97	12.98	+0.01
	48.41	45.57		12.96	−0.01
	27.12	25.61		12.97	0.00
	35.79	33.73		12.95	−0.02
	34.57	32.27		12.82	−0.15
	30.46	28.14		12.69	−0.28
	46.17	43.70		13.00	+0.03
N - metbyl - *N* - *p*-tolylbenzenesulfonamide	33.73	30.78	12.28	12.53	+0.25
	41.71	36.86		12.15	−0.13
N - methyl - *p* - aminophenol sulfate	30.38	21.10	9.64	9.54	−0.10
	30.24	21.70		9.85	+0.21
Sulfanilamide Analysis by macromethod (7) gave 18.68, 18.69, 18.72, and 18.73% sulfur, average 18.71%	33.92	45.26	18.73	18.32	−0.41
	30.33	40.69		18.43	−0.30
	30.35	40.92		18.52	−0.21
	33.60	45.52		18.61	−0.12
	31.14	42.90		18.92	+0.19
	30.89	41.37		18.40	−0.33
	36.54	49.71		18.69	−0.04
	34.21	45.76		18.37	−0.36
	20.57	28.02		18.71	−0.02
	30.60	40.96		18.39	−0.34
	34.73	46.77		18.50	−0.23
	36.09	49.11		18.69	−0.04
Thiocarbanilide (m. p. 152–3° observed)	31.85	32.39	14.03	13.97	−0.06
Thiourea	32.00	97.92	42.11	42.03	−0.08
	27.75	84.86		42.00	−0.11
$(CH_3)_2CHCH.S.C_4H_9$ | $CONH_2$	32.23	40.07	16.95	17.07	+0.12
p-$CH_3C_6H_4.S.CHC_2H_5$ | $COOC_2H_5$	25.35	24.59	13.46	13.63	+0.17
	19.79	19.82		13.72	+0.26
$C_2H_5SO_2CH_2CONH_2$	25.66	40.20	21.21	21.51	+0.30
	35.99	54.91		20.96	−0.25
	36.64	56.79		21.34	+0.13
	37.72	57.99		21.11	−0.10
Bis - [*p,p'* - (*N*-methyl - *N* - benzenesulfonyl)] - diphenylmethane	39.39	36.86	12.67	12.85	+0.18
	31.35	29.10		12.74	+0.07
Methyl - *p* - tolylsulfone	33.15	46.36	18.85	19.21	+0.36
	37.19	51.79		19.12	+0.27
Bis-(phenylsulfonyl)-methane	29.36	47.81	21.70	22.36	+0.66
	40.45	64.34		21.85	+0.15
Taurine Purity 98.8% based on Kjeldahl analysis for nitrogen (10 results averaged 11.07%)	35.15	64.53	(25.62)	25.22	Purity calcd. from sulfur 98.6%
	32.05	58.83		25.22	
	22.67	41.81		25.33	

TABLE III. SEMIMICRODETERMINATION OF SULFUR IN ORGANIC COMPOUNDS

(By use of sodium peroxide bomb, with procedure modified to provide for removal of silica introduced by decomposition in presence of glass ampoule)

Compound	Sample	$BaSO_4$	Sulfur		
			Calcd.	Found	Difference
	Mg.	*Mg.*	%	%	%
Solid Substances					
Sulfanilamide	39.96	53.62	18.73	18.43	−0.30
	40.30	54.83		18.54	−0.19
	31.09	41.67		18.41	−0.32
N - *p* - tolylbenzenesulfonamide	31.39	29.64	12.97	12.93	−0.04
	40.44	38.67		13.13	+0.16
	43.51	41.42		13.08	+0.11
Liquid Substances					
p-$CH_3C_6H_4.S.CHC_2H_5$ (COOC$_2$H$_5$)[a]	18.32	18.11	13.46	13.57	+0.11
	15.59	15.34		13.52	+0.06
	31.78	31.23		13.50	+0.04
Thiophene[b], redistilled synthetic, b. p. 85° observed	37.59	102.52	38.13	37.48	−0.65
	27.05	74.21		37.68	−0.45
	24.42	67.09		37.74	−0.39
	16.52	45.23		37.61	−0.52
	15.54	42.35		37.43	−0.70

[a] This compound is a liquid but is not sensibly volatile, and need not be weighed in glass ampoule. Results in Table II were obtained with samples weighed directly into bomb cup from Lunge-Rey pipet.

[b] Results for thiophene average about 0.5% too low, owing presumably to volatilization of undecomposed thiophene into space above charge during ignition.

in hastening the granulation of precipitated barium sulfate in semimicroanalysis, and to determine whether or not the presence of picric acid adversely affects the accuracy of the method. The results of these tests are listed in Table IV.

Table IV indicates that when picric acid is used the precipitate may safely be collected after digestion for an hour, but that in the absence of picric acid a digestion period of 1 hour, or even of 2 hours, is insufficient and leads to negative error. It is shown also that precipitates of semimicro size may not be ready for filtration in 10 to 15 minutes, as was shown by Lindsly (*19*) to be permissible in macroanalysis. The results (one good, two low but passable, one very low) obtained after such early filtration suggest that to ensure consistently satisfactory analyses a longer digestion period is advisable. When the precipitate is allowed to stand overnight before filtration, satisfactory results are obtained whether or not picric acid is present. There is no clear indication that by allowing the liquid and precipitate to stand overnight the chloride inclusion is decreased and the composition of the precipitate improved, as reported for the macromethod by Johnston and Adams (*10*), or that the presence of picric acid slightly increases coprecipitation of barium chloride (*19*). The procedure may not be sufficiently accurate to show a consistent noticeable response to these influences, which may also be compensatory. In most of the analyses reported in Tables II and III picric acid was used and the precipitate was allowed to stand with the liquid overnight before filtration.

In accuracy, precision, and range of applicability the procedure described may be considered satisfactory, and on the whole it compares well with the macro- and micromethods. Tables II and III list the unselected results of analyses made independently a year apart by the two junior authors. Table IV includes results which probably represent the method at its best. Accuracy is affected by relatively small manipulative irregularities, as the manipulations are those of the macromethod executed on a reduced scale.

Summary

This paper describes a Parr sodium peroxide bomb assembly suitable for the decomposition of semimicrosamples of organic compounds, a gravimetric semimicroprocedure for determination of sulfur in organic compounds by combustion in the semimicrobomb, and a procedure for removal of silicic acid introduced when liquid samples in glass ampoules are decomposed in the sodium peroxide bomb.

Tests indicate that in the semimicromethod the digestion period required to render the precipitate of barium sulfate filterable is shortened when precipitation is effected in the presence of picric acid.

TABLE IV. EFFECT OF PICRIC ACID IN SEMIMICROPRECIPITATION OF BARIUM SULFATE

(Substance analyzed: *N*-*p*-tolylbenzenesulfonamide. Sulfur, calculated, 12.97%)

Picric Acid	Time of Digestion	Sample	$BaSO_4$	Sulfur	
				Found	Error
	Min.	*Mg.*	*Mg.*	%	%
Yes	10–15	27.35	30.37	12.37	−0.60
		32.52	34.91	12.79	−0.18
		28.30	29.99	12.96	−0.01
		29.65	32.12	12.68	−0.29
	Hours				
Yes	1	41.04	38.75	12.97	0.00
No		35.97	33.00	12.60	−0.37
		30.41	27.87	12.58	−0.39
Yes	2	35.44	33.47	12.97	0.00
		30.24	28.47	12.93	−0.04
No		29.74	27.44	12.68	−0.29
		29.84	27.56	12.69	−0.28
Yes	Overnight	31.15	29.30	12.92	−0.05
		39.05	37.18	13.08	+0.11
No		33.09	31.17	12.94	−0.03
		35.61	33.52	12.93	−0.04

Acknowledgment

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Experimental work has been started leading to the extension of the use of the semimicrobomb to the determination of halogens, phosphorus, and arsenic in organic compounds.

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Determination of Cleanliness of Feather and Down Bedding Materials

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FEATHERS and downs used in articles of bedding and furniture are obtained from geese, ducks, and chickens, the principal geographic sources being China, Eastern Europe, and the United States. When plucked from the fowl, such materials contain bits of skin, flesh, blood, and other extraneous decomposable organic matter.

Under the provisions of the New York State Bedding Law and Rules (*9*), feathers and downs must be properly cleaned before being sold by processors for use in pillows, comforters, cushions, and other articles of bedding and furniture. Rule 1 (e) (*9*) specifies that tests for cleanliness be made in accordance with the following procedure:

> Allow a one-half ounce sample of the material to stand in one quart of freshly boiled but cold water for at least two hours, and then thoroughly agitate the material. Strain off the water through clean muslin into a clean container. There shall be no visible sediment or cloudiness and no cloudiness or offensive odor shall develop in the wash water when allowed to stand for seventy-two (72) hours in an unsealed container at room temperature not below seventy (70) degrees Fahrenheit.

Cleanliness tests of the same type are used by some other states, and are enumerated in purchase specifications of the Federal Government (*3*) and the City of New York (*8*).

The long period of time needed to obtain results with the above test, together with difficulties encountered in interpreting the results in terms of cloudiness and odor, showed the need for chemical tests which could be made in a few hours and which would be more indicative of the cleanliness of such materials.

Free and Combined Ammonia

Since ammonia is formed in the natural decomposition of organic matter, it occurred to the authors that more ammonia and water-soluble ammonia compounds should be present in unwashed materials, and therefore serve as an indicator of the relative amounts of decomposing organic matter present in washed and unwashed feathers and downs.

To determine the ammonia content, the procedure was almost identical with that employed in the micromethod used by this laboratory for detecting second-hand cotton (*5*, *6*) and kapok (*7*) filling materials, the ammonia being finally liberated in a modified Parnas and Wagner micro-Kjeldahl distilling apparatus.

Five grams of feathers and down are boiled for 20 minutes in 200 ml. of water containing 1 ml. of 0.1 *N* hydrochloric acid, then filtered and washed with hot water. The combined filtrate and washings are evaporated on a hot plate to a volume of 4 to 6 ml. The residue is washed into a graduated Kramer-Gittelman tube, cooled, and diluted with water to 12.5 ml.

A 5-ml. aliquot is pipetted into a large culture tube, 250 × 25 mm. in diameter, 5 drops of caprylic alcohol are added to prevent foaming, and then 7 ml. of saturated borax solution. The culture tube is immediately inserted into the steam-distilling apparatus (Figure 1), and the liberated ammonia is distilled over through a water-cooled block-tin condenser into a 50-ml. Erlenmeyer flask containing 10 ml. of 2 per cent boric acid and 5 drops of methyl red indicator (0.05 per cent methyl red in 95 per cent ethyl alcohol). The culture tube is kept in the apparatus for 5 minutes after the top of the tin condenser has become too hot to touch. During the last 2 minutes the Erlenmeyer flask is lowered so that the end of the condenser is above the boric acid solution.

The solution is titrated with 0.01 *N* sulfuric acid, using a microburet, until the color is a deep pink. It is then heated to boiling and the titration is continued until a full red color is reached. The number of milliliters of sulfuric acid used is noted.

A blank determination is made using water and the reagents alone. The milliliters of sulfuric acid obtained with the blank are subtracted to get the corrected volume of sulfuric acid.

Calculations: Ml. of H_2SO_4 (corrected) × 0.0085 = % of NH_3 in sample

Oxygen Consumed

The second chemical test used was the Knight test (*4*), with certain modifications. This is a test for oxygen consumed and serves to indicate the relative amounts of oxidizable matter present in washed and unwashed materials.

Ten grams of feathers and down are transferred to a large Mason jar and 1 liter of water at room temperature is added. The material is thoroughly wetted by shaking and placed in an incubator at 25° C. for one hour. It is then filtered through 19-cm. coarse filter paper, and 100 ml. of filtrate are pipetted into a porcelain casserole. The liquid is made just acid to litmus with 6 *N* sulfuric acid and 1 ml. of acid is added in excess. The solution is titrated in the cold with 0.1 *N* potassium permanganate by adding 2-drop portions and then stirring until a pink color persists for 60 seconds.

The number of milliliters of potassium permanganate used is noted, and calculated to number of grams of oxygen per 100,000 grams of sample (oxygen number).

1 ml. of 0.1 *N* $KMnO_4$ = 0.8 mg. of oxygen = 80, oxygen number.

Chlorides

A study of the chloride test (*1*, *2*) as a cleanliness indicator had been planned. However, preliminary analyses of well waters used by some processors for washing feathers showed very high sodium chloride contents. This indicated that further work on the chloride test would be valueless and therefore the study was discontinued.

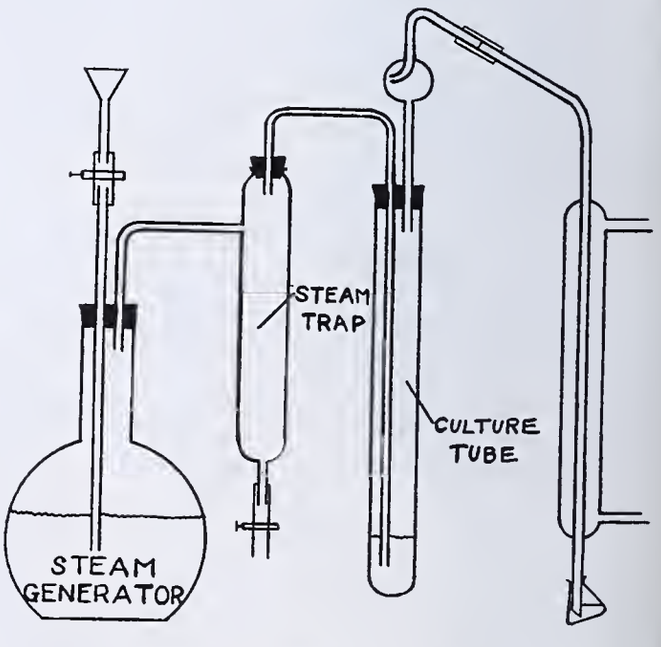

FIGURE 1. DISTILLING APPARATUS

Samples Used

In order to obtain materials for analysis, visits were made to factories of six of the largest feather processors in this country, where feathers and downs of various types were washed and dried in the presence of the authors.

The raw feathers (sometimes after a preliminary dusting) were agitated for approximately 20 minutes in a washing solution consisting of water and a small amount of detergents. The dirty wash solution was then drained off, and the feathers were given 2 or more rinsings by agitating them in plain water for periods of 10 to 20 minutes each, draining after each rinsing. The wet feathers were centrifuged for approximately 10 minutes to remove most of the remaining water, and then transferred to a steam-jacketed drying chamber, where they were agitated by rotating paddles while a current of warm air passed through them and carried off the residual moisture.

A total of 86 samples of feathers and downs was obtained; 43 were unwashed materials; 43 consisted of the same materials after washing and drying. These materials, sampled before washing and after washing and drying, were of the following types:

Type	No. of Samples
Chicken, butcher, domestic	12
Duck, China	13
Duck, Long Island	3
Goose, China	9
Goose, European	6
	43

Results of Analyses

Each sample was analyzed by the oxygen-consumed test, the ammonia test, and the present cleanliness test (Table I).

The results of the oxygen test are reported as "oxygen numbers", the oxygen number of a sample being the number of parts (by weight) of oxygen consumed during the test, calculated to 100,000 parts of sample. The results of the ammonia test are reported as the per cent of water-soluble ammonia found. The results of the present cleanliness test are reported in the routine terminology of this test.

The data contained in Table I are summarized in Table II, which lists the number of samples giving various analytical results. Six samples (Long Island duck feathers) were not included in this summary as it was found best to deal separately with them. Table II, then, summarizes the results obtained on analysis of 80 samples: 40 samples of unwashed materials, and 40 samples of the same materials after washing and drying.

Interpretation of Results

Present Cleanliness Test. Table II shows (column 4) that none of the unwashed material had a clear solution, regardless of odor. At first sight it might seem that material showing a clear solution could be classed as properly washed. However, 22.5 per cent of the washed materials had a slight cloudiness, and therefore would be classed by this standard as improperly washed.

If materials showing a cloudy solution were rated as "not properly clean", 15 per cent (column 5) of the unwashed materials would be passed as properly washed.

The presence of a cloudy solution is shown to be a much better indicator of improper washing than a bad odor; 85 per cent of the unwashed material had a cloudy solution, whereas only 55 per cent had a bad odor.

Oxygen-Consumed Test. All the unwashed materials had oxygen numbers above 20.

Of the washed materials 95 per cent had oxygen numbers below 20. The remaining 5 per cent consisted of two materials, both washed by the same concern. All the materials washed by the other five concerns had oxygen numbers below 20.

Two samples of washed materials had been treated with ozone immediately after washing and drying; their oxygen numbers were well below 20 in spite of this treatment.

On the basis of this test, feathers and downs having oxygen numbers above 20 could be classed as "not properly clean".

Ammonia Test. All the unwashed materials had water-soluble ammonia contents above 0.020 per cent, and all the washed materials had

Table I. Analytical Results

Description	Present Cleanliness Test: Before Washing Solution	Before Washing Odor	After Washing Solution	After Washing Odor	Oxygen Number: Before washing	Oxygen Number: After washing	Per Cent of Ammonia: Before washing	Per Cent of Ammonia: After washing
Chicken Feathers, Domestic, Butcher								
Brown and gray	Cloudy	Bad	Slightly cloudy	Slight	103	8	0.129	0.009
	Cloudy	Bad	Clear	O. K.	99	8	0.146	0.009
	Cloudy	Bad	Slightly cloudy	Slight	133	8	0.170	0.009
	Cloudy	Bad	Clear	O. K.	178	8	0.221	0.008
	Cloudy	Bad	Clear	O. K.	124	8	0.102	0.011
	Cloudy	Bad	Clear	O. K.	90	8	0.115	0.009
Brown quilled	Cloudy	Bad	Clear	Slight	120	27[a]	0.185	0.018[a]
	Cloudy	Bad	Clear	Slight	122	25[a]	0.191	0.018[a]
Gray	Cloudy	Bad	Clear	O. K.	92	8	0.149	0.018
White	Cloudy	Bad	Clear	O. K.	176	8	0.085	0.010
	Cloudy	Bad	Clear	O. K.	54	8	0.132	0.009
	Cloudy	Bad	Clear	O. K.	80	8	0.069	0.011
Duck Feathers and Down								
Gray China	Cloudy	Bad	Clear	O. K.	38	8	0.043	0.006[b]
	Cloudy	Slight	Clear	Slight	29	8	0.081	0.011[b]
	Cloudy	Bad	Slightly cloudy	Slight	72	8	0.120	0.014
	Slightly cloudy	Slight	Slightly cloudy	Slight	38	16	0.088	0.009
	Cloudy	Bad	Slightly cloudy	O. K.	40	8	0.062	0.008
	Cloudy	Bad	Clear	O. K.	36	8	0.072	0.010
	Cloudy	Slight	Clear	O. K.	32	8	0.076	0.008
	Cloudy	Slight	Clear	O. K.	28	8	0.064	0.003[c]
	Slightly cloudy	Slight	Clear	O. K.	36	8[d]	0.065	0.010
	Cloudy	Slight	Clear	O. K.	38	8	0.087	0.010
White L. I.	Clear[e]	O. K.	Clear	O. K.	8[e]	8	0.013	0.008[b,e]
	Slightly cloudy[e]	Slight	Clear	O. K.	10[e]	8	0.014[e]	0.006[b,e]
	Slightly cloudy[e]	Slight	Clear	O. K.	8[e]	8	0.017[e]	0.006[b,e]
Gray China	Slightly cloudy	Bad	Slightly cloudy	O. K.	28	8	0.089	0.010
	Cloudy	Slight	Slightly cloudy	O. K.	40	8	0.044	0.007
	Cloudy	Slight	Slightly cloudy	O. K.	32	8	0.049	0.004
Goose Feathers and Down								
Gray China	Cloudy	Bad	Clear	Slight	40	8	0.026	0.007[b]
	Cloudy	Slight	Clear	O. K.	68	8	0.068	0.010
	Cloudy	Slight	Clear	O. K.	28	8	0.086	0.009
	Cloudy	Slight	Clear	O. K.	30	8	0.065	0.009
	Cloudy	Slight	Clear	O. K.	28	8	0.103	0.009
	Slightly cloudy	Slight	Clear	Slight	28	8	0.085	0.008
White China	Slightly cloudy	Slight	Clear	O. K.	37	7	0.024	0.009[b]
	Slightly cloudy	O. K.	Clear	O. K.	34	8	0.059	0.011
	Cloudy	Slight	Clear	Slight	32	8[d]	0.064	0.012
White Hungarian	Cloudy	Bad	Clear	O. K.	101	8	0.067	0.005[b]
	Cloudy	Bad	Clear	Slight	103	8	0.064	0.006[b]
	Cloudy	Slight	Clear	O. K.	27	8	0.049	0.009
	Cloudy	Bad	Clear	Slight	92	12	0.058	0.012
	Cloudy	Bad	Slightly cloudy	O. K.	40	8	0.037	0.005
	Cloudy	Slight	Clear	O. K.	38	8	0.052	0.005

[a] These two samples washed by same concern.
[b] Ammonia-solution used as one of detergents in initial washing step.
[c] Final rinsing in ammonia-solution.
[d] Treated with ozone immediately after washing and drying.
[e] Long Island duck feathers are washed to some extent before being sold to processors.

TABLE II. SUMMARY OF ANALYSES OF 80 SAMPLES

Present Cleanliness Test, Rule 1(e) Solution	Odor	Before Washing: Chicken feathers (butcher) (twelve samples)	Duck feathers and down (thirteen samples)	Goose feathers and down (fifteen samples)	Combined all types (forty samples)	Percentage of all types (100%)	After Washing: Chicken feathers (butcher) (twelve samples)	Duck feathers and down (thirteen samples)	Goose feathers and down (fifteen samples)	Combined all types (forty samples)	Percentage of all types (100%)
Clear	O. K.	..	..	..	0	0	8	6	10	24	60
Clear	Slight	..	..	..	0	0	2	1	4	7	17.5
Clear	Bad	..	..	..	0	0	..	..	..	0	0
Slightly cloudy	O. K.	..	..	1	1	2.5	..	4	1	5	12.5
Slightly cloudy	Slight	..	2	2	4	10	..	2	..	2	5
Slightly cloudy	Bad	..	1	..	1	2.5	2	..	..	2	5
Cloudy	O. K.	..	..	..	0	0	..	..	..	0	0
Cloudy	Slight	..	6	7	13	32.5	..	..	..	0	0
Cloudy	Bad	12	4	5	21	52.5	..	..	..	0	0
Oxygen Number											
Under 20		..	..	..	0	0	10	13[a]	15[a]	38	95
20–49		..	12	11	23	57.5	2[b]	..	..	2	5
50–99		5	1	2	8	20	..	..	..	0	0
100 and over		7	..	2	9	22.5	..	..	..	0	0
Ammonia, %											
0.000–0.009		..	..	..	0	0	6	7[c,d]	11[d]	24	60
0.010–0.019		..	..	..	0	0	6	6[b]	4	16	40
0.020–0.049		..	2	4	6	15	..	..	..	0	0
0.050–0.099		2	10	10	22	55	..	..	..	0	0
0.100 and over		10	1	1	12	30	..	..	..	0	0

[a] One sample of goose feathers and one sample of duck feathers treated with ozone immediately after washing and drying.
[b] Two of these samples washed by same concern.
[c] One sample received final rinsing in ammonia solution.
[d] In 5 samples of duck feathers and 4 samples of goose feathers, ammonia solution used as one of detergents in initial washing step.

ammonia contents below 0.020 per cent. The ammonia contents of the washed materials were less than 0.020 per cent even in the 10 cases where ammonia solution was used in the processing as one of the washing or rinsing agents.

If this test were used as a standard, feathers and downs having water-soluble ammonia contents above 0.020 per cent could be classed as "not properly clean".

RELATIVE EFFICIENCIES OF THREE TESTS. Table II shows a significant correlation between the results of the oxygen and ammonia tests. Each of the 40 unwashed materials had an oxygen number above 20 and an ammonia content above 0.020 per cent. Similarly, 95 per cent of the 40 washed materials had both an oxygen number below 20 and an ammonia content below 0.020 per cent. If both these tests were used as standards, feathers and downs having either ammonia contents above 0.020 per cent or oxygen numbers above 20 could be classed as "not properly clean".

As an aid in comparing the oxygen and ammonia tests with the present cleanliness test, Table III was prepared. It enumerates all the results possible with the present test, groups the number of samples which gave each result, and lists the maximum and minimum oxygen numbers and per cents of ammonia found in the materials which comprise each group.

TABLE III. COMPARISON OF AMMONIA AND OXYGEN TESTS WITH PRESENT CLEANLINESS TEST[a]

Present Cleanliness Test	Unwashed Samples: No. of samples	Oxygen number range	Per cent ammonia range	Washed Samples: No. of samples	Oxygen number range	Per cent ammonia range
Clear solution						
Odor O. K.	None	..	..	23	6–8	0.003–0.018
Odor slight	None	..	..	8	8–26	0.006–0.018
Odor bad	None	..	..	None	..	..
Slightly cloudy solution						
Odor O. K.	1	34	0.059	5	8	0.004–0.009
Odor slight	4	28–38	0.065–0.087	4	8–16	0.008–0.014
Odor bad	1	28	0.088	None	..	..
Cloudy solution						
Odor O. K.	None	..	..	None	..	..
Odor slight	13	27–68	0.048–0.103	None	..	..
Odor bad	21	36–178	0.026–0.206	None	..	..

[a] Like Table II, does not include 6 samples of L. I. duck feathers.

The data presented in Table III show that the presence of a slight cloudiness has no significance in telling whether materials are washed or unwashed. Materials having a slight cloudiness had oxygen numbers varying widely from 8 to 38 and ammonia contents ranging from a low of 0.008 per cent to a high of 0.088 per cent.

The relative efficiencies of the three tests are compared in Table IV.

TABLE IV. RELATIVE EFFICIENCIES OF THREE TESTS

Test Used	Best Standard Found for Designating Material as "Not Properly Clean"	On Basis of This Standard: Unwashed materials detected	Washed materials rated "not properly clean"
		%	%
Present cleanliness	Cloudy solution	85	None
Oxygen consumed	Oxygen number above 20	95	5
Ammonia	Ammonia content above 0.020 per cent	100	None

Long Island Duck Feathers

Long Island duck feathers are washed to some extent before being sold to the feather processors, and therefore do not properly fall into the category of completely unwashed feathers from a cleanliness viewpoint. Accordingly, they were not included in Tables II, III, and IV. This viewpoint was borne out by the results presented in Table V.

Table V shows that the three samples of duck feathers, as received by the feather processors, contained much less decomposing and oxidizable matter than most of the completely unwashed materials. In fact, these three materials before washing by the processors would be classed as "properly clean" by all three cleanliness tests, according to the standards used in Table IV. However, one sample had an ammonia content of 0.017 per cent, which is close to the ammonia standard of 0.020 per cent. Therefore, samples of this type of duck feather might easily show an ammonia content greater than 0.020 per cent when received by the processors.

Table V. Long Island Duck Feathers

(Before and after washing by processors)

Present Cleanliness Test				Oxygen Number		Per Cent of Ammonia	
Before Washing Solution	Odor	After Washing Solution	Odor	Before washing	After washing	Before washing	After washing
Clear	O. K.	Clear	O. K.	8	8	0.013	0.008
Slightly cloudy	Slight	Clear	O. K.	10	8	0.014	0.006
Slightly cloudy	Slight	Clear	O. K.	8	8	0.017	0.006

Summary

The ammonia test was found to be the best of the three cleanliness tests. Using 0.020 per cent water-soluble ammonia as a dividing line, it detected all the unwashed materials in the 40 cases of this type enumerated in Table II. The use of ammonia solution as washing or rinsing agent did not interfere with this test in any of the ten cases tried. The test showed good possibility of indicating in some cases that Long Island duck feathers are not sufficiently clean, when received by processors, to use without further washing.

The oxygen-consumed test was second-best for detecting unwashed material. Using an oxygen number of 20 as the dividing line, it detected 95 per cent of the unwashed materials in the 40 cases enumerated in Table II. Treating washed feathers with ozone did not interfere with the oxygen test, in the two cases so treated. The test showed little possibility for use with Long Island duck feathers.

The present cleanliness test rated last place. Using a "cloudy solution" as a dividing line, it detected only 85 per cent of the unwashed materials in the 40 cases enumerated in Table II, and showed little possibility for use with Long Island duck feathers.

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Microdetermination of Molecular Weights of Dark-Colored Organic Materials

Cryoscopic Method

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THE cryoscopic method for the microdetermination of molecular weights involves measuring the depression of the melting point (*4*), the freezing point (*1*), or both the melting and freezing points (*6*) of some pure solvent. Since the freezing points of many available solvents, and of the solutions of organic materials in these solvents, can be measured more accurately than can the corresponding melting points, the freezing point procedure is used exclusively in this laboratory.

This procedure, which also requires less time and manipulation than do any of the above-mentioned cryoscopic procedures, has been used successfully by the writer in determining the molecular weights of ester gums and dark-colored resins. Molecular weight determinations on ester gum are useful, along with other analytical data, in estimating the proportions of mono-, di-, or tri-esters present. In the case of resins, the molecular weight values give an indication of the degree of condensation. Some resinous materials, however, form solutions too dark to permit accurate observation of the freezing points by the regular freezing point procedure.

This difficulty has been overcome by placing a small glass capsule (Figure 1) in the molecular weight tube with the unknown and solvent. This capsule is made by placing a lead shot (BB) in the bottom of the molecular-weight tube and sealing off at a distance of 10 to 12 mm. from the bottom. The lead shot keeps the capsule submerged in the solution. The annular space thus formed between this capsule and the molecular-weight tube contains only a very thin layer (0.5 to 1 mm. in thickness) of the solution, in which the first appearance of crystals, upon cooling, can be readily detected.

A device, constructed of aluminum, which facilitates thorough mixing of the melted sample and solvent without their removal from the hot oil bath, is shown in Figure 2—above, the normal position for a determination; below, the tubes tilted through approximately 180° to permit thorough mixing of the contents. The molecular-weight tubes are held in place by spring steel sections *s* and *s'*, which can be adjusted to hold tubes of different lengths. The entire device fits easily into a 300-cc. culture flask.

Procedure

In carrying out an actual determination, 8 to 11 mg. of the unknown and 80 to 130 mg. of a pure grade of camphor, or some other suitable solvent (*5*), are weighed directly in a very thin-walled molecular-weight tube, the capsule is inserted, and the tube is carefully sealed (Figure 1). Duplicate determinations can be carried out simultaneously. The oil bath, which is maintained 5° to 10° above the melting point of the pure solvent until a thoroughly mixed liquid solution of sample and solvent is obtained, is cooled 1° C. per minute and the freezing point of the solution in the molecular-weight tubes is observed. The point at which the crystals first appear can be more easily observed with the aid of a hand lens. This procedure is repeated using the same solutions, and the average of the values thus obtained is reported as the freezing point of the solutions. The molecular weight of the unknown is then calculated by the equation:

$$\text{Mol. wt.} = \frac{W \times K \times 1000}{S \times (t_1 - t_2)}$$

where W is the weight of solute (unknown); S is the weight of solvent; t_1 and t_2 are the freezing temperatures of the pure solvent

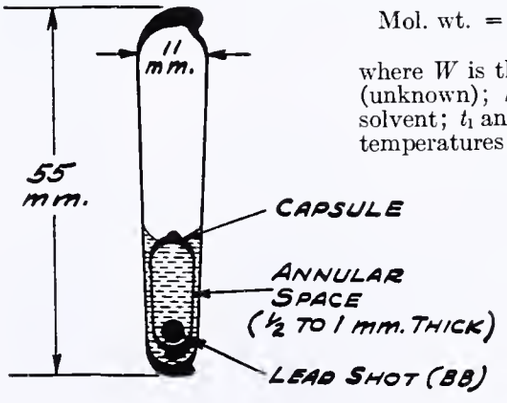

Figure 1. Modified Freezing Point Tube

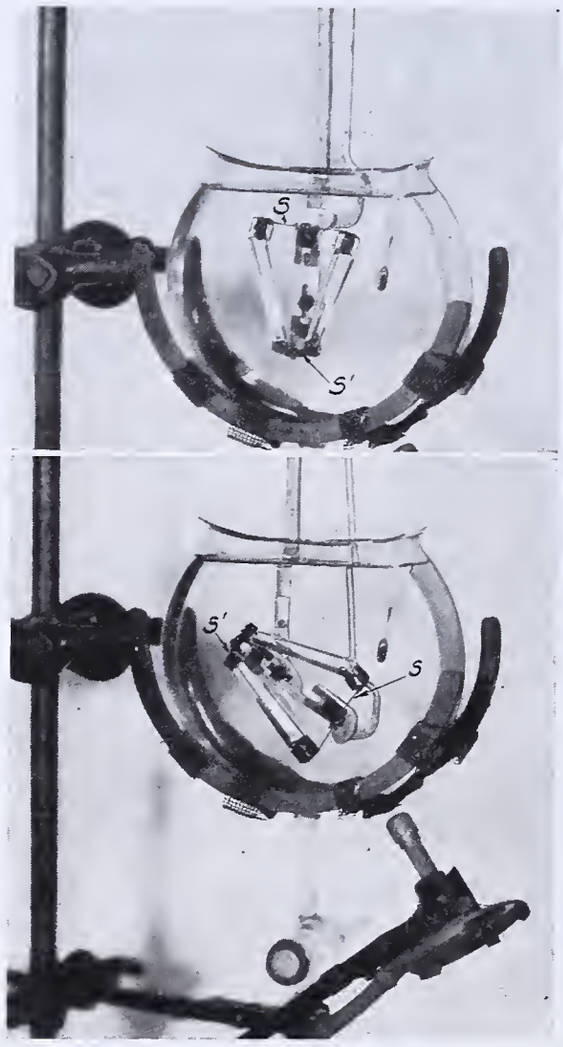

FIGURE 2. DEVICE FOR MIXING CONTENTS OF MOLECULAR WEIGHT TUBES

Normal position above, tilted position below

and solution, respectively; and K is the molal freezing point lowering, or cryoscopic constant, of the pure solvent, obtained by the above procedure with a pure compound of known molecular weight, such as benzoic acid.

The cryoscopic constants and freezing points reported below for camphor and borneol were determined by this procedure. Benzoic acid, obtained from the National Bureau of Standards, was used as the solute in determining the cryoscopic constants. Since these constants have been found to vary with the source of the solvent (*6*), as well as with the method used for their determination (*2, 3*), they should always be determined, as should the freezing points, on each lot of cryoscopic solvent by exactly the same procedure that is to be used subsequently for the determination of molecular weights.

	Camphor		
	Lot 1	Lot 2	Borneol
Freezing point, ° C.	176.0	176.8	205.1
Cryoscopic constant, K	40.0	39.6	34.9

The application of the cryoscopic method to the microdetermination of molecular weights of organic materials presupposes certain definite knowledge of the behavior of the material (solute) under question in the presence of the solvent selected: The solute must not decompose at or near the freezing temperature, t_1, of the solvent; there must be no reaction between the solute and solvent at this temperature; there must be no association of the molecules of the solute in the solvent; and the molal freezing point constant, K, must be independent of the concentration of the solute.

In Table I are reported molecular-weight values obtained by the freezing point procedure using the device described above for dark-colored organic materials. All weighings were made on a semimicrobalance, sensitive to 0.02 mg.

Summary

A device is described which permits the microdetermination of molecular weights of dark-colored organic materials by measuring the depression of the freezing point of a pure solvent. This procedure, which has been used in this laboratory for the past 2 years, ensures the same accuracy, about 5 per cent, as that obtained by measuring the depression of the melting point of the solvent. The freezing point procedure

TABLE I. MOLECULAR-WEIGHT MICRODETERMINATIONS BY THE CRYOSCOPIC METHOD

Wt. of Solute *Mg.*	Wt. of Solvent *Mg.*	Freezing Point Depression ° C.	Molecular Weight Found Camphor	Borneol	Theoretical Molecular Weight	Error %
			Retene			
9.01	113.96	13.0	243	...	234.3	+3.7
8.17	102.94	12.9	246	...	..	+5.0
8.11	125.78	11.2	228[a]	...	..	−2.7
8.46	122.04	12.3	223[a]	...	..	−4.8
8.02	106.92	11.6	...	226	..	−3.5
7.79	89.43	14.0	...	217	..	−7.4
			Hydroquinone Dimyristyl Ether			
9.50	106.45	7.3	489	...	502.8	−2.7
9.88	105.34	7.8	481	...	..	−4.3
8.64	93.66	7.2	512	...	..	+1.8
8.36	93.97	7.0	508	...	..	+1.0
9.23	108.46	6.6	516	...	..	+2.6
11.15	108.42	8.1	508	...	..	+1.0
9.23	109.37	7.0	477[a]	...	..	−5.1
8.70	128.44	5.4	497[a]	...	..	−1.2
9.23	133.41	6.1	473[a]	...	..	−5.9
			Tetrabromophenolsulfonphthalein			
9.41	109.05	5.2	657[a]	...	670.0	−1.9
8.45	101.89	5.1	644[a]	...	..	−3.9
9.43	112.47	4.9	678[a]	...	..	+1.2
9.25	112.73	5.0	650[a]	...	..	−3.0
10.82	122.60	5.5	635[a]	...	..	−5.2
			1-Naphthol Benzein			
8.61	119.65	7.5	380[a]	...	392.4	−3.2
8.94	127.02	7.4	377[a]	...	..	−3.9
8.55	115.07	7.6	387[a]	...	..	−1.4
8.91	126.09	7.5	373[a]	...	..	−4.9
9.58	117.60	7.1	...	400	..	+1.9
8.44	116.06	6.2	...	409	..	+4.2
9.00	125.25	6.3	...	398	..	+1.5
8.40	108.32	6.6	...	410	..	+4.5
			Vinsol[b], Dark Resin			
10.89	109.88	10.0	396	...	..	...
9.19	101.66	9.1	397	...	..	...
12.45	109.37	9.7	...	410	..	...
11.03	102.49	9.0	...	417	..	...
			Vinsol[b]-Treated, Dark Resin			
10.15	100.54	6.6	612	...	..	...
10.18	102.12	6.8	586	...	..	...
			Vinsol[b] Fraction, Dark Resin			
9.90	105.06	4.6	819	...	..	...
9.76	100.76	4.9	791	...	..	...
			Ester Gums			
10.16	101.68	4.2	952	...	..	...
9.83	99.52	4.3	919	...	..	...
8.90	105.19	4.1	825	...	..	...
8.76	107.09	4.0	818	...	..	...
9.44	100.81	3.9	960	...	..	...
9.09	100.09	3.8	956	...	..	...
8.87	103.06	3.3	1043	...	..	...
9.11	104.10	3.4	1030	...	..	...
8.71	98.70	3.8	929	...	..	...
9.51	102.94	4.1	901	...	..	...

[a] Camphor from lot 2 used as solvent.

[b] Registered in U. S. Patent Office by Hercules Powder Co. Vinsol is essentially a gasoline-insoluble pine wood resin.

requires less time and manipulation than does the melting point procedure.

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Determination of Zinc by Precipitation as Zinc Anthranilate

A Gravimetric Semimicromethod

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THE need for a more rapid and accurate method for the determination of zinc in tin-lead solder led to the investigation of the application of anthranilic acid, in view of the high sensitivity of the reaction involved. A method formerly in use required at least 4 hours for a determination on solder, while the recovery of zinc was always less than the total zinc present.

Funk and Ditt (*2*) have shown that zinc can be quantitatively precipitated by the sodium salt of anthranilic acid when it is added to a neutral solution or one faintly acid with acetic acid. Precipitation takes place from a dilute solution at room temperature. Zinc anthranilate is crystalline and may be easily filtered. The sensitivity of the precipitation reaction is 1 in 1,000,000. Anthranilic acid, which may be used for analytical purposes, can be purchased from the Eastman Kodak Company, Rochester, N. Y.

The literature contains no method describing the use of anthranilic acid for determining zinc in white metal alloys.

Interfering Elements

Among the metals which may cause interference by coprecipitating to a marked extent are copper and iron. The concern is chiefly with copper, as iron causes no interference unless more than 10 mg. is present. The effect of iron upon the determination of zinc is shown in Table I. The data were obtained by taking various quantities of pure iron and pure zinc which were carried through as described below.

Copper, which reacts with sodium anthranilate like zinc (*2*), can cause interference even when present in minute amount. Funk and Ditt (*3*) describe its accurate precipitation and determination. The sensitivity in the precipitation reaction between the copper and anthranilate ions is the same as with zinc.

Of the splendid methods described in the literature for accurately separating copper from certain elements, including zinc, two are especially suitable. Copper can be completely separated from all the other elements present in all kinds of white metal alloys, in the form of a black, finely divided suspension of metallic copper from a sodium hydroxide solution, by addition of hydrazine hydrochloride (*4*). The copper is filtered off and the zinc determined in the filtrate.

Microquantities of copper, in the range from 0.05 to about 50 micrograms of copper, can be determined accurately by a colorimetric method, using the very sensitive organic reagent dithizon (*1*) and the Keil colorimeter.

The present method may be used for the determination of semimicroquantities of zinc, present as impurity in tin-lead solder in amount from 0.01 to 0.10 per cent, and for quantities of about 8 per cent in zinc-tin alloys.

The procedure, with certain modifications, can be used for determining zinc in other material of greater zinc content—for example, the familiar fluxing agent consisting of a large quantity of zinc chloride with a small amount of ammonium chloride. It may also be used for the determination of zinc oxide impregnated in a certain type of enamel, coating the inside surface of tin cans (Table II) in which various foods are packed, notably corn and meats.

The time necessary to carry out a zinc-tin analysis is 1 hour, about 2 hours for solder, 1.5 hours for zinc chloride soldering salts, and 1.5 to 2 hours for the zinc oxide content of enamel coating.

The accuracy of the method is shown in Table III, giving data obtained by analyses of a sample of flux, and in Table IV, in which several of the analyses were carried out consecutively, in duplicate.

Solutions and Materials Required

A saturated solution of pure bromine in concentrated hydrochloric acid is prepared by shaking vigorously in a glass-stoppered bottle 12 ml. of bromine in 100 ml. of acid.

Sodium tartrate.

Sodium anthranilate reagent, made by weighing 3 grams of anthranilic acid into a beaker and adding about 20 ml. of water and 22 ml. of *N* sodium hydroxide. Finally the solution is made neutral or faintly acid by adjustment with 2 per cent acetic acid, filtered, and made up to volume in a 100-ml. flask.

Gooch crucibles of about 10-ml. capacity.

Procedure

SOLDER AND ZINC-TIN ALLOYS. Dissolve the sample in bromine–hydrochloric acid, 0.5 gram for solder and 0.05 gram for zinc-tin alloys. When solution of the sample is complete add 2

TABLE I. EFFECT OF IRON UPON ZINC DETERMINATIONS

Zinc Added	Iron Added	Weight of Precipitate	Zinc Found
Mg.	*Mg.*	*Mg.*	*Mg.*
1.40	0.10	7.30	1.41
1.80	6.25	9.00	1.74
0.20	10.00	1.05	0.20
0.30	20.00	55.50	10.75
0.14	15.00	41.10	7.96
0.11	5.00	0.60	0.116
0.115	8.00	0.60	0.116

TABLE II. ZINC DETERMINATION IN ENAMEL COATINGS FROM TIN CANS

(Area of sample, 6.452 sq. cm., 1 square inch)

Film Weight of Sample	Weight of Precipitate	Zinc Added	Total Zinc Present	Zinc Found	Recovery
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	%
5.5	5.60			1.08	
5.5	6.40	0.15	1.23	1.24	99.0

Table III. Determinations on a Sample of Zinc Chloride Soldering Salts

(Zinc added, 0.30 mg. Total zinc present, 18.26 mg.)

Aliquot Taken from 200-Ml. Volume	Weight of Sample	Weight of Precipitate	Zinc Found	Recovery
Ml.	*Gram*	*Mg.*	*Mg.*	*%*
20	0.08209	185.2	35.82	
10	0.041045	92.8	17.975	
10	0.041045	92.7	17.96	
10	0.041045	92.7	17.96	
10	0.041045	94.1	18.23	99.8

Table IV. Zinc Determination in White Metal Alloys

Sample	Weight of Sample	Zinc Added	Total Zinc Present	Weight of Precipitate	Zinc Found	Recovery
	Gram	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*%*
Solder	0.50			1.45	0.28	
	0.50	0.08	0.36	1.85	0.36	
	0.50			0.90	0.17	
	0.50	0.35	0.52	2.70	0.52	
	0.50			1.30	0.25	
	0.50	0.15	0.40	2.10	0.41	102.5
	0.25			3.30	0.64	
	0.25			3.30	0.64	
	0.25	1.05	0.74	3.85	0.75	101.3
	0.50			10.80	2.09	
	0.50			10.90	2.11	
	0.50	0.175	2.29	11.90	2.30	100.4
Solder sample containing 5.23 per cent of iron	0.50			1.50	0.29	
	0.50	0.175	0.47	2.40	0.47	
Zinc-tin alloy	0.05			22.20	4.30	
	0.05			22.20	4.30	
	0.05	0.40	4.70	24.20	4.69	99.8
Synthetic mixtures of pure tin, lead, and zinc		0.30		1.55	0.30	
		0.50		2.60	0.504	100.7
		4.00		20.80	4.03	100.7
		0.675		3.40	0.669	98.6
		6.00		31.10	6.02	100.4
		5.55		29.00	5.62	101.2
		6.50		33.60	6.50	

to 3 ml. of concentrated sulfuric acid and evaporate to fumes. Lead, which usually is not present in zinc-tin alloys, is filtered after suitable dilution, and washed with 2 per cent sulfuric acid wash solution. Dissolve in the filtrate 0.3 gram of sodium tartrate to hold tin in solution. Neutralize the solution to litmus, first adding a saturated solution of sodium hydroxide drop by drop and concluding the neutralization with powdered sodium carbonate, adding a slight excess. Add 2 per cent acetic acid until the solution is neutral or faintly acid. Add to the cold solution 25 ml. of the reagent slowly with constant stirring. Allow the solution to stand for 30 minutes or longer for complete precipitation of zinc.

Filter through a weighed Gooch crucible, washing the precipitate with a solution containing one part of reagent to 15 parts of water by volume. Wash out the excess reagent by two or three washings with alcohol. Dry the crucible in the oven at 100° to 105° C. for 30 minutes, cool, weigh, and calculate the zinc content. Zinc anthranilate contains 19.37 per cent of zinc.

Semimicroquantities of Zinc in Presence of Large Amounts of Iron. In the presence of large amounts of iron semimicroquantities of zinc can be accurately determined by a procedure described in the literature (*5*), which is a modification of the basic acetate separation of iron. It can be used to separate very large quantiites of iron, as much as 0.5 gram and higher, quantitatively from elements present as impurities in alloy steels and minerals.

White Metal Alloys. Dissolve a 0.5-gram sample in bromine–hydrochloric acid. When solution is complete add 1 gram of potassium chloride to the acid solution of the metallic chlorides. Evaporate to apparent dryness, break up the residue, and heat at 100° C. until the odor of hydrochloric acid is very faint (5 to 7 minutes). In the presence of potassium chloride, it is possible to evaporate the solution to dryness without decomposition of ferric chloride. Enough hydrochloric acid remains to give a clear solution when the residue is treated with 20 ml. of water and boiled gently.

Dissolve 3 grams of sodium acetate in 100 ml. of water and make the solution neutral to litmus with the addition of 1 per cent acetic acid. Add the sodium acetate to the ferric chloride solution, dilute to 400 ml., heat just to boiling, and hold at this temperature for 1 minute. The basic acetate soon precipitates. Filter the solution while hot, and wash the precipitate with hot 1 per cent ammonium acetate solution. Continue the washing until the volume is 600 ml. Transfer the filtrate to an 800-ml. beaker, add 5 ml. of concentrated sulfuric acid, and evaporate the solution down to fumes. Just before fumes of sulfuric acid appear add 5 ml. of concentrated nitric acid to destroy interfering ammonium salts. Continue evaporation to dense fumes of sulfuric acid. Cool, wash into a 150-ml. beaker, and evaporate to a volume of 15 ml. Neutralize, first adding a saturated sodium hydroxide solution, then powdered sodium carbonate until alkaline, and finally make slightly acid by adding a few drops of 2 per cent acetic acid. Add 0.3 gram of sodium tartrate, following the regular procedure, and finally precipitate zinc by adding 25 ml. of reagent.

This is an excellent method for separating large quantities of iron from small amounts of zinc, as there is neither adsorption nor coprecipitation of zinc. The iron content of the solder sample shown in Table IV was found to be 5.23 per cent, a quantity much too large to enable determination of zinc according to the regular procedure without removal of iron. The zinc content of the sample is 0.058 per cent. The recoveries are proof of sufficient precision and accuracy.

Zinc Chloride Fluxes. Take any convenient weight of sample, dissolve in water containing a few drops of hydrochloric acid, and make up to volume in a volumetric flask. Measure off aliquots accurately in a 150-ml. beaker, using a buret of 10-ml. capacity, neutralize the solution to litmus with powdered sodium carbonate, add the anthranilic acid reagent, and continue as in the procedure for white metal alloys. Calculation of the amount of zinc chloride from the value obtained for zinc, carried out in duplicate, shown in Table III gives 91.20 per cent. These experiments also prove precision and accuracy to be entirely sufficient.

Enamel Coating of Tin Cans. Cut a disk with an area of 12.9 sq. cm. (2 sq. inches), and remove the enamel film from the whole piece, or cut the disk into two 6.452-sq. cm. (1-sq. inch) pieces, using one of them for the determination. The film weight is obtained by weighing the section before and after removal of the film. Immerse the sample in a few milliliters of chloroform in a 50-ml. beaker for rapid removal of the enamel coating. Evaporate the chloroform cautiously to avoid loss of small portions of the film. Add 1 to 2 ml. of concentrated sulfuric acid, heat over a small flame, and add a few drops of Superoxol to destroy organic matter. Cool and add 10 ml. of water and 0.10 to 0.20 gram of sodium tartrate. Allow to dissolve and continue as in the other procedures.

Synthetic Mixtures of Tin, Lead, and Zinc. Suitable standard samples of known zinc content were not available as a means for determining accuracy. Therefore pure tin and lead were taken in the same proportion as in the type of solder most widely used—i. e., 40 per cent of tin and 60 per cent lead—together with various added quantities of pure zinc, carefully measured from a solution of a zinc salt.

The data obtained by these analyses, shown in Table IV, give proof of the accuracy.

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Analysis of Cellulose Derivatives

Determination of Total Combined Acyl in Cellulose Organic Esters

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Most of the published methods for the determination of combined acyl in cellulose derivatives have been restricted to acetyl in cellulose acetate. The three best methods, Eberstadt, alcoholic alkali, and Ost acid distillation, have been further investigated as to their applicability for general acyl analysis. The effects of their more important variables have been measured, and the precision, accuracy, and range of applicability of each are reported.

In order to avoid errors due to the heterogeneity of the reaction mixture and to excess alkalinity or acidity, temperature, and time, these variables must be controlled within definite limits. It was found that saponifications by the Eberstadt method are best run at an initial alkali concentration of 0.25 *N* for 48 hours at not higher than 35° C. This is the most accurate method, but is applicable to cellulose acetate and only certain other esters. Saponification methods using alcoholic alkali are applicable to practically all cellulose esters, but are less accurate and reliable. The most satisfactory conditions are 0.25 *N* initial concentration for 24 hours at not higher than 30° C. The acid distillation method of Ost is restricted in its application, but it has special uses due to shorter elapsed time required for an analysis and to the fact that only volatile acidity is measured.

THE increasing studies of the various esters of cellulose and the ever-widening field of application of these esters to practical uses have increased the need for tested and reliable methods for the analysis of these products. It is the purpose of this paper to present the results of accumulated experience and of special studies made on the most satisfactory and generally applicable of the methods available.

The methods for the determination of total combined acyl in cellulose organic esters fall into two general classes: saponification with alkaline reagents or decomposition and hydrolysis by acids. The methods in the literature, applying principally to the determination of acetyl or combined acetic acid in cellulose acetate, have been well reviewed by Krueger (*8*) and by Murray, Staud, and Gray (*11*), and recent additional references are given by Marsh and Wood (*9*).

Of the saponification methods using aqueous alkali (*8*), the one devised by Eberstadt (*4*) working with Knoevenagel (*6*, *7*) and modified somewhat by Murray, Staud, and Gray (*11*) has proved useful and reliable when applied to cellulose acetate and to certain similar esters of low molecular weight organic acids. It breaks down, however, when applied to esters of the type of cellulose stearate and even to certain cellulose butyrates. This method involves swelling the sample with warm aqueous alcohol, followed by addition of aqueous alkali and a long saponification at room temperature.

The use of sodium ethylate was proposed by Cross and Bevan (*3*), and was successfully applied with modifications by Woodbridge (*15*) and Mork (*10*). Methyl or ethyl alcoholic alkali was used similarly by Green and Perkin (*5*). These methods are capable of yielding good results if conditions of time, temperature, and alkali strength are carefully chosen. Furthermore, these alkalies attack a much wider range of cellulose esters than aqueous alkali. Zemplen (*16*) saponified cellulose acetate with traces of sodium methylate in refluxing absolute methyl alcohol, but his method is not applicable to quantitative analysis because the amount of alkali consumed is not stoichiometric.

Rapid methods involving solution of the sample in pyridine have been described by Battegay and Penche (*2*) and also by Murray, Staud, and Gray (*11*). Aqueous alkali is added to the solution and it is heated for a half hour at not more than about 55° C. Roeper (*14*) has tried various other solvents, including acetone, acetone-water, and acetone-alcohol mixtures with aqueous alkali at various times and temperatures. Concordant and reasonable results were obtained with certain esters, but these methods are not generally applicable nor accurate.

The acid hydrolysis method was proposed in 1906 by Ost (*12*), who suggested the use of strong sulfuric acid followed by steam-distillation of the liberated acetic acid, and also by Green and Perkin (*5*), who decomposed the ester with sulfuric acid in the presence of absolute ethyl alcohol and then distilled off ethyl acetate and saponified this ester in the distillate. Various modifications of these methods have been reported (*8*), but nearly all this work has been restricted to the determination of combined acetic acid. Abribat (*1*) dissolved and degraded cellulose acetate with cold concentrated hydrochloric acid and then hydrolyzed off the acetyl groups by diluting the acid. The acetic acid liberated was titrated potentiometrically in the presence of hydrochloric acid. Pilgrim (*13*) used warm strong hydrochloric acid to degrade the cellulose, and hydrolyzed the acetyl groups by progressive dilution of the acid under conditions mild enough to avoid charring. The reaction mixture was then diluted to a known volume and a suitable aliquot titrated. The difference in titer between the sample and a blank was taken as a measure of the acetic acid liberated.

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These methods have the following inherent difficulties or objections:

1. Most of the methods, and particularly the practical and widely applicable ones, involve heterogeneous conditions for the saponification or hydrolysis. The physical form of the solid ester is thus an important variable. It is often necessary to powder or reprecipitate the sample before reproducible and reliable results can be obtained. Since insoluble regenerated cellulose is formed in all these methods except the strong acid procedure, the final titration must be slow enough to allow for complete soaking out of the excess reagent. These factors may cause low results due to slow rate of penetration of the reagent and incomplete reaction, or high results due to slow soaking out of the excess reagent.
2. When this first difficulty is met by dissolving the ester in a solvent, such as pyridine or acetone, the method is limited to esters soluble in such solvents and whose solutions will tolerate the addition of a comparatively large volume of aqueous or alcoholic alkali. Not all the esters of cellulose will meet these requirements. The back-titration must still be made in the presence of regenerated cellulose.
3. Cellulose and its derivatives form acidic decomposition products when heated excessively with alkalies or acids in the presence of air. Strong alkalinities and elevated temperatures must be avoided, and the variables of time, temperature, and reagent concentration must be balanced carefully if accurate results are to be obtained.
4. The precision of these methods is, of course, easily measurable, but the accuracy is very difficult to establish. The heterogeneous conditions met in saponification and back-titration and the difficulty of complete distillation of acids after acid decomposition tend to produce low results if the conditions are not vigorous enough, while too severe conditions of reagent concentrations and temperature produce high results. Furthermore, it is extremely difficult to prepare a cellulose ester of known acyl content to serve as a standard. Simpler esters, such as ethyl acetate or a glucose acetate, cannot be used for this purpose, since they do not duplicate the heterogeneous conditions met in the analysis of cellulose esters.

These difficulties can be met by using tested procedures within limits of reagent concentration, time, and temperature which have been shown to be satisfactory. By taking these precautions, high results can be avoided. Low results are usually caused by lumpy or hard sandy precipitates which resist penetration by the reagent, and can be eliminated by powdering the sample, or preferably by reprecipitating from suitable solvents to get a soft fluffy product. The accuracy of a method can be determined by comparing the best results from one method with those by other methods after the variables and limitations of each have been studied. Long experience with standard types of cellulose esters, particularly the acetates, enables one to establish fairly accurate acyl contents for each type, and these materials can then be used as reference samples, if not for primary standards. By combining this information, practical limits of accuracy can be set up.

In the following sections, three methods which have proved satisfactory are given in detail with results of studies of the effects of their most important variables. The precision and accuracy attainable and the limits of applicability are also given for each method.

Eberstadt Method

Procedure. Accurately weighed 1-gram samples of the thoroughly dried ester are placed in 250-ml. Erlenmeyer flasks, and 40 ml. of 75 per cent ethyl alcohol are added to each. Alcohol denatured by the 3-A formula may be diluted for this purpose. The flasks are then heated loosely stoppered for 0.5 hour at 50° to 60° C. (A double-walled bath containing refluxing methyl alcohol in the jacket provides the right amount of heat for this purpose.) Then 40 ml. of 0.5 *N* sodium hydroxide solution are added and the flasks are heated for 15 minutes at 50° to C. Blanks containing alcohol and alkali are run with each set of samples or at least on new reagents. The flasks are stoppered tightly and allowed to stand at room temperature for 24, or preferably 48, hours with occasional swirling. At the end of this time the excess alkali is back-titrated with standard 0.5 *N* hydrochloric acid, using phenolphthalein indicator, and an excess of about 1 ml. of acid is added. The alkali is allowed to soak out from the regenerated cellulose for several hours and more acid is added if necessary. Finally alkali or acid is added to establish the exact neutral point, and the per cent acyl is calculated somewhat as follows:

$$[(\text{ml. of acid for blank}) - (\text{ml. of acid for sample})] \times (\text{acid normality}) \frac{(\text{equivalent wt.})}{\text{sample wt.} \times 10} = \% \text{ acyl}$$

Most of the results given in the tables which follow were calculated to acetyl or to "apparent acetyl" (equivalent weight 43) even though some samples were known to contain other acyl groups.

When a large number of samples is to be analyzed by this method, time and labor can be saved by dispensing the alkali from a dispensing pipet which delivers approximately 40 ml. Almost all the acid can be added from a dispensing pipet which delivers approximately 20 ml., and the titration can be

Table I. Effect of Time of Swelling at 50° to 60° C.

Sample	Apparent Acetyl for Various Times of Swelling: None	15 min.	30 min.	1 hour	3 hours	5 hours
	%	%	%	%	%	%
Cellulose acetate 1	40.5	40.6	40.7	40.7	40.9	40.5
	40.6	40.6	40.7	40.7	40.9	40.5
Cellulose acetate propionate 1	40.5	40.6	40.5	40.7	40.9	40.6
	40.5	40.6	40.5	..	40.9	40.6
Cellulose acetate 3	43.7	43.6	43.7	..	43.6	..
	43.2	..	43.5	..	..	..

Table II. Effect of Alkali Concentration

Sample	Time	Apparent Acetyl at Various Effective Alkali Concentrations: 0.25 *N*	0.5 *N*	1 *N*
	Hours	%	%	%
Cellulose acetate 1 (40.5% acetyl)	2.5	28.4	40.3	..
		33.7	39.1	..
	4	40.0	40.7	..
		40.2	40.4	..
	6	38.9	39.9	41.0
		40.7	40.1	40.9
	16	40.5	40.8	42.3
		40.5	41.0	42.6
	24	40.5	40.9	43.4
		40.7	41.0	43.5
	48	40.5	41.2	44.9
		40.5	41.2	45.0
	72	40.5	..	..
		40.5	..	..
Cellulose acetate 2 (40.5% acetyl)	2.5	27.8	40.5	..
		29.3	40.5	..
	4	38.4	40.6	..
		39.5	38.8	..
	6	37.6	40.0	41.0
		39.9	40.1	41.5
	16	38.9	40.8	41.9
		40.7	41.5	..
	24	40.5	40.9	43.4
		40.5	..	43.0
	48	40.5	41.2	44.8
		40.5	41.2	..
	72	40.5	..	..
		40.5	..	..
Cellulose acetate propionate 1 (40.5% apparent acetyl)	2.5	34.7	39.4	..
		35.5	40.2	..
	4	40.1	40.5	..
		40.1	40.5	..
	6	39.4	39.9	40.8
		38.9	40.1	..
	16	39.8	39.9	41.3
		40.7	40.5	41.6
	24	40.6	40.8	43.4
		40.7	40.9	43.4
	48	40.4	41.2	41.2
		40.6	41.2	41.7
	72	40.4	..	..
		40.4	..	..
Cellulose acetate butyrate 1 (35.5% apparent acetyl)	2.5	24.8	32.3	..
		28.5	34.5	..
	4	34.1	35.3	..
		34.7	35.4	..
	6	29.8	32.8	38.2
		34.0	..	38.8
	16	35.0	35.8	35.9
		35.5	35.4	36.8
	24	35.5	35.4	35.9
		35.5	35.4	..
	48	35.5	35.5	39.6
		35.5	35.5	37.8
	72	35.7	..	..
		35.8	..	..

finished using ordinary burets. Only the difference between the buret readings for the blank and the sample titration enters into the calculation.

EFFECT OF TIME OF SWELLING. The effect of the time of swelling at 50° to 60° C. is shown in Table I. The above procedure was followed and only the time of swelling was varied as indicated.

These data show that the time of swelling is not important, and in many cases this presoak could be eliminated. It does not add much to the manipulation and does no harm, however, so it is a precaution well worth taking and is to be recommended.

EFFECT OF ALKALI CONCENTRATION. The same procedure was again followed except that the alkali normality and the time of standing were varied as indicated in Table II. The solutions added were actually 0.5, 1.0, and 2.0 *N*, but since they were diluted with equal volumes of 75 per cent alcohol, their effective normalities at the start of the saponification were half these values.

The accepted values for the acetyl or apparent acetyl contents—i. e., all the acidity calculated to acetyl—of the various esters are given in the first column. Comparison of these accepted values with the other data leads to the following conclusions:

At an effective normality of 0.25, the accepted acetyl value is reached in 16 to 24 hours' reaction time, and this value is not raised even after 72 hours.

At an effective normality of 0.5, the accepted value is reached in about 16 hours, but after 24 hours the value observed rises slowly above this accepted figure.

At an effective normality of 1, abnormally high values are obtained even at short reaction times, and the precision is poorer than at lower alkalinities.

The most satisfactory conditions for use in this procedure are saponification at an effective initial normality of 0.25 for not less than 24 hours and preferably for 48 hours. A set of 24 samples taken at random was analyzed using these conditions; a 24-hour saponification was sufficient for 18 samples, but 48 hours was required to get acceptable results on the other six. Consequently it is safest to allow 48 hours for all samples.

EFFECT OF TEMPERATURE. The same samples were tested by this method at several different temperatures and with varying reaction times to measure the effect of temperature on the accuracy of the results. In all cases 0.5 *N* alkali was added, making a resultant initial concentration of 0.25 *N*, and the 50° to 60° C. heat treatment was omitted in the reactions run at 0° C. The data obtained are given in Table III.

The results of these experiments indicate the following conclusions:

Saponifications at 0° C. are incomplete and the results erratic for reaction times of less than 24 hours. Acceptable results were obtained on three of these four samples using saponification times of 24, or preferably 48, hours.

Reactions run smoothly at room temperature, and accurate values are obtained in from 24 to 72 hours. There is apparently no tendency toward high results when the specified conditions of temperature and alkalinity are met.

When the saponifications are run at an elevated temperature such as 60° C. very erratic results are obtained in short reaction times, and very high results are obtained after about 10 hours. This result is the same as is caused by too high alkalinity.

In the previous section it was shown that the most satisfactory conditions of alkalinity and time were 0.25 *N* and 24 to 48 hours. These experiments show that room temperature (25° to 30° C.) is satisfactory, but temperatures above about 35° C. are to be avoided.

FORMATION OF ACIDS OTHER THAN ACETIC ACID. The apparent acetyl values in Tables II and III show that too high values are obtained when the alkalinity and temperature exceed the specified conditions. A special experiment was run to prove that this extra acidity is due to acids other than acetic.

TABLE III. EFFECT OF TEMPERATURE

Sample	Time	Apparent Acetyl for Various Reaction Temperatures 0° C.	30° C.	40° C.	60° C.
	Hours	%	%	%	%
Cellulose acetate 1 (40.5% acetyl)	1	30.4	28.4	..	26.0
		33.1	26.2	..	31.9
	2.5	37.8	28.4	..	35.5
		36.6	33.7	..	35.6
	4	34.0	40.0	..	36.1
		35.8	40.2	..	39.7
	6	34.6	40.0	..	42.0
		34.1	40.2	..	40.4
	18	34.1	40.5	40.5	42.7
		31.7	40.5	40.5	41.8
	24	40.4	40.5	40.6	43.4
		40.4	40.7	40.6	42.9
	48	40.4	40.5	40.9	44.9
		40.5	40.7	40.9	45.6
	72	..	40.5	40.9	..
		..	40.5	41.1	..
Cellulose acetate 2 (40.5% acetyl)	1	31.8	29.7	..	31.3
		37.4	29.5	..	33.0
	2.5	34.1	27.8	..	38.2
		..	29.3	..	37.7
	4	38.5	38.4	..	39.5
		39.4	39.5	..	38.5
	6	35.2	39.9	..	41.1
		32.4	37.6	..	41.0
	18	32.4	38.9	40.4	43.0
		37.3	40.7	40.4	42.7
	24	40.0	40.5	40.5	43.5
		40.9	40.5	40.5	43.5
	48	40.4	40.5	40.7	44.1
		40.4	40.5	40.7	44.2
	72	..	40.5	40.8	..
		..	40.5	40.8	..
Cellulose acetate propionate 1 (40.5% apparent acetyl)	1	31.1	33.8	..	35.4
		33.5	31.0	..	36.5
	2.5	32.5	34.7	..	38.3
		33.8	35.5	..	34.8
	4	36.9	40.0	..	39.7
		39.1	40.1	..	39.1
	6	33.5	38.9	..	40.3
		39.4	..	..	41.2
	18	37.5	40.7	40.2	41.6
		36.9	39.8	40.6	41.8
	24	40.4	40.6	40.3	42.2
		40.4	40.7	40.4	42.0
	48	40.7	40.4	40.5	43.7
		40.4	40.6	40.5	43.4
	72	..	40.4	..	..
		..	40.4	..	..
Cellulose acetate butyrate 1 (35.5% apparent acetyl)	1	17.0	21.9	..	31.1
		17.6	21.6	..	24.7
	2.5	25.3	28.5	..	32.9
		16.4	24.8	..	35.2
	4	25.1	34.7	..	36.1
		23.7	34.1	..	35.8
	6	25.6	29.8	..	37.3
		..	34.0	..	37.2
	18	24.5	35.0	35.2	38.7
		..	35.5	35.3	38.3
	24	33.0	35.5	35.5	38.6
		31.1	35.5	35.5	38.6
	48	33.3	35.6	35.6	..
		33.7	35.6	35.6	..
	72	..	35.7	..	..
		..	35.7	..	..

Cellulose acetate 1 (40.5 per cent acetyl content) was saponified with 1 *N* sodium hydroxide for 48 hours at a temperature of 55 to 60° C. resulting in an apparent acetyl value of 46.0. Other samples which had received this same heat treatment, together with samples saponified under normal conditions to give an apparent acetyl value of 40.5 per cent, were then acidified with phosphoric acid, and the volatile acids present were isolated by vacuum distillation. Isolation of the volatile acids by the technique used is known to be about 98 per cent quantitative and, in order to eliminate errors in calculation resulting from incomplete distillation, the volatile acids from a sample saponified under normal conditions were distilled simultaneously with the acids from the sample having a high acetyl value. Aliquots of the distillates were titrated and the volatile acids were calculated to per cent apparent acetyl. The acetyl values of the samples saponified normally and under extreme conditions averaged 39.4 and 40.7 per cent, respectively. This difference represents volatile acidity produced by the strong conditions of heating and alkalinity.

Portions of the distillates were gently refluxed with yellow mercuric oxide, a reagent which does not decompose acetic acid. Titration of the samples thus treated resulted in apparent acetyl values of 39.4 per cent for both samples, indicating that the excess volatile acids had been destroyed by gentle oxidation.

Formic acid and other oxidizable acids in the distillates were also measured by the reducing action of the distillate on mercuric chloride. The mercurous chloride precipitate formed by the

TABLE IV. EFFECT OF ALKALI CONCENTRATION AT 25° C.

Sample	Time	Apparent Acetyl at Various Alkali Concentrations 0.25 *N*	0.5 *N*	1 *N*
	Hours	%	%	%
Cellulose acetate 1 (40.5% acetyl)	6	40.1	40.6	40.5
		40.2	..	40.2
	16	40.5	40.5	40.6
		40.5	40.5	40.6
	24	40.5	40.3	40.7
		40.5	40.8	40.7
	48	40.6	40.8	41.2
		40.6	41.0	40.9
	72	40.8	41.3	..
		40.8	40.9	..
Cellulose acetate butyrate 1 (35.5% apparent acetyl)	6	34.8	35.3	35.2
		34.8	35.3	35.2
	16	35.6	35.5	35.5
		35.6	35.6	..
	24	35.6	35.7	35.6
		..	35.7	35.6
	48	35.6	35.8	35.7
		35.6	35.8	35.6
	72	35.6	36.0	..
		35.6	37.8	..
Cellulose acetate stearate (25.6% apparent acetyl)	6	25.0	25.1	24.6
		24.6	25.1	24.4
	16	25.5	25.3	25.0
		25.5	25.4	24.8
	24	25.6	25.4	25.5
		25.7	25.3	25.3
	48	26.2	25.7	25.5
		26.2	25.6	25.5
	72	26.0	26.5	..
		26.7	25.6	..
Cellulose caprate (23.2% apparent acetyl)	6	15.0	22.4	22.0
		15.4	22.4	22.0
	16	22.0	22.6	22.7
		22.1	22.6	22.6
	24	23.2	22.7	23.0
		23.2	22.7	23.0
	48	23.3	23.2	23.9
		23.3	23.2	23.8
	72	23.3	23.7	..
		23.2	23.3	..

sample saponified under normal conditions corresponded to 0.15 per cent acetyl and that detected in the sample saponified under extreme conditions corresponded to 0.88 per cent acetyl.

From this experiment it was concluded that about one fourth of the excess acidity was due to volatile acids, about three fourths was due to the formation of nonvolatile acid groups, and the excess acidity due to volatile acids was caused by easily oxidizable acids, probably largely formic acid. Thus unless conditions of saponification are controlled within established limits, acids other than acetic acid may be formed and the results will be abnormally high.

LIMITS OF APPLICABILITY. The modified Eberstadt method, as described above, has proved to be the most accurate, reliable, and satisfactory of all the methods tested. It is applicable to the analysis of all cellulose acetates and other cellulose esters of relatively low molecular weight organic acids. When this method is applied to esters of the homologous series of fatty acids, it is satisfactory for the acetates, propionates, and some of the butyrates. It is unsatisfactory and gives low results for the valerates, caproates, caprates, stearates, etc. It is satisfactory for acetate propionates and for most acetate butyrates, but is unsatisfactory for acetate stearates. The limits of applicability cannot be drawn sharply because the physical condition of the sample is very important.

PRECISION AND ACCURACY. When all the variables of the method are properly controlled, results may be expected having precision of ±0.1 per cent apparent acetyl or ±0.25 per cent of the numerical value, and accuracy of ±0.2 per cent apparent acetyl or ±0.5 per cent of the numerical value obtained.

As a further check on the accuracy of the method when it is in everyday use, it is advisable to run a check batch with every set of samples.

Alcoholic Alkali Method

PROCEDURE. Half-gram samples of the thoroughly dried ester are carefully weighed and transferred to 250-ml. Erlenmeyer flasks. Forty milliliters of a 0.25 *N* solution of sodium hydroxide in 95 per cent ethyl alcohol are pipetted into each flask, and other flasks with reagent only are carried along as blanks and for standardizing the alkali. This also avoids errors due to changes in volume of the alcoholic solution with temperature. If desired the reagent can be made up 0.5 *N* and 20-ml. aliquots used. Then 20 ml. of alcohol are added from a graduate to produce the same effective normality. The flasks are stoppered and set aside for from 16 to 24 hours at not higher than 30° C. At the end of this time the excess alkali is back-titrated with standard 0.25 or 0.5 *N* hydrochloric acid, and a slight excess is added. After allowing about 4 hours for the alkali to soak out, the excess acid is titrated with 0.5 *N* sodium hydroxide to a phenolphthalein end point, taking the precautions required in the Eberstadt procedure. The amount of acid added is corrected for the alkali required to establish the end point, and the result is calculated by the equation used for the Eberstadt procedure.

EFFECT OF ALKALI CONCENTRATION. A series of analyses was run in which the above procedure was followed except that the strength of the alcoholic alkali and time of reaction were varied as indicated in Table IV. Twenty-milliliter aliquots of the 1 *N* solution were used to reduce the back-titration.

These data show that the saponification is complete in from 16 to 24 hours using 0.25 or 0.5 *N* alkali. Normal alkali tends

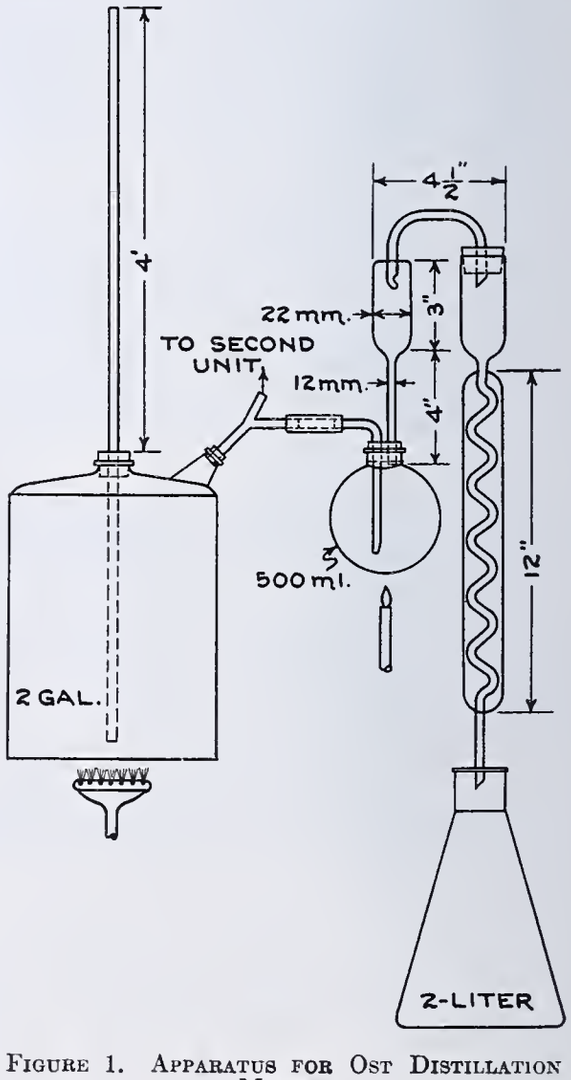

FIGURE 1. APPARATUS FOR OST DISTILLATION METHOD

to give high results in some cases. Use of 0.25 *N* alkali is recommended because it is preferable from the standpoint of ease of manipulation and its keeping qualities are better. The samples tested vary greatly in composition and demonstrate the wider applicability of this method.

Effect of Temperature. The data in Table V show the effect of temperature using a 16-hour saponification time. Temperatures of 25° to 30° C. give satisfactory results, but at 40° C. the results tend to run high.

Other Alkalies. Sodium methylate or sodium ethylate, prepared by treating anhydrous alcohol with metallic sodium, react very incompletely with cellulose esters at ordinary temperature unless there are small amounts of water present. Since water must be added in some form, it is more practical to dissolve sodium hydroxide in 95 per cent ethyl alcohol than to bother with metallic sodium. Sodium hydroxide is more soluble in 95 per cent ethyl alcohol than in methyl alcohol of the same strength.

Limits of Applicability. This method, unlike the modified Eberstadt method described above, is not limited to the lower aliphatic acid esters, but is generally applicable to all cellulose esters at least up to and including cellulose stearate.

Precision and Accuracy. Precision of ±0.2 per cent apparent acetyl or ±0.5 per cent of the numerical value obtained is attainable by this method if sufficient care is taken with the back-titration. An accuracy of ±0.4 per cent apparent acetyl or ±1.0 per cent of the numerical value or better is usually obtained when strict attention is paid to condition of the samples, hydrolysis time and temperature, and exact location of the end point. The range given for the accuracy attainable has been set wider than indicated by the data in Tables IV and V because many samples show poorer stability toward alkali than those reported, and there is often an upward drift in observed acyl contents with reaction times from 16 to 72 hours. This drift obscures the true value and reduces the accuracy.

Table V. Effect of Temperature

Sample	Apparent Acetyl for Various Reaction Temperatures			
	20° C.	25° C.	30° C.	40° C.
	%	%	%	%
Cellulose acetate 1	40.7	40.5	40.5	40.9
	40.7	40.5	40.7	40.9
Cellulose acetate butyrate 1	35.4	35.6	35.6	36.1
	35.4	35.6	35.6	36.1
Cellulose acetate stearate	25.6	25.6	25.6	26.6
	25.6	25.7	25.6	26.6
Cellulose caprate	15.2	22.4	22.8	23.7
	15.2	22.4	22.8	23.7

Ost Distillation Method

Many variations of this method are known, and most of them can be made to yield acceptable results after experience has been acquired. The procedure described below uses simple apparatus and the manipulation is also comparatively simple.

Procedure. Hard or coarse materials should first be ground or powdered. Then 0.3-gram samples of the dried material are accurately weighed and transferred to 500-ml. round-bottomed flasks, 10 ml. of sulfuric acid (1 to 1 by volume) are added to each flask, and the flasks are stoppered and shaken gently to wet the entire sample. The flasks are set aside stoppered at room temperature until an hour or more after the samples are entirely in solution. Then 50 ml. of carbon dioxide–free distilled water are added to each, and the flasks are connected to distillation assemblies shown in Figure 1.

The volatile acids are distilled with steam at the rate of about 600 ml. of distillate per hour until 1500 or 1600 ml. have been collected. The distilling flask is heated to maintain the volume approximately constant during the distillation. The rate of distillation is very important, for too rapid a rate results in the mechanical carry-over of small amounts of sulfuric acid. The large volume of distillate is required to get complete distillation of acetic acid. The entire distillate is titrated with 0.1 *N* sodium hydroxide solution using phenolphthalein indicator. A blank distillation and titration are run and a correction is applied when necessary. After the titration has been completed, a few milliliters of barium chloride solution are added to prove the absence of sulfates. If a cloudiness is observed, the titration result is questionable. The alkali consumed is calculated to acetyl.

The apparatus, as shown in Figure 1, consists of a steam generator, a 500-ml. round-bottomed flask, a distilling head and trap, a condenser, and a receiver. The design of the distilling head is critical, for the rate of distillation and the amount of distillate collected must be balanced so that the organic acids are distilled completely without carrying over any sulfuric acid. This is best accomplished by repeatedly analyzing a cellulose ester of known composition or a weighed sample of pure sodium acetate and varying the above conditions if necessary until accurate results are obtained. Once the technique has been mastered, satisfactory results can be obtained consistently.

Effect of Sulfuric Acid Concentration. Sulfuric acid diluted 1 to 1 by volume is strong enough to dissolve most cellulose ester samples in a reasonable length of time without charring. A solution of two parts of acid to one of water is also satisfactory if charring can be avoided, but it is usually preferable to use the more dilute solution to avoid errors from this cause. Solutions more dilute than 1 to 1 require too much time for solution of the sample and may give incomplete reaction. The recommended concentration (1 to 1) produces colorless solutions, and hydrolysis times have been varied from 3 to 16 hours without changing the results.

Effect of Apparatus Design. As mentioned above, the apparatus design, and particularly the design and dimensions of the distilling head, are important. Too small a head or too rapid a rate of distillation results in a carry-over of sulfuric acid, while too large a head requires the collection of too large a volume of distillate. A good condenser is needed, and the spiral type is recommended.

Effect of Volume of Distillate. The distillation curve for acetic acid (per cent distilled *vs.* volume of distillate) under the conditions chosen can be measured by distilling the acid from a 0.25-gram sample of pure sodium acetate and titrating the distillate portionwise. Data thus obtained showed 100 per cent distillation for a distillate volume of 1250 ml., 90 per cent at 700 ml., and 80 per cent at 400 ml. By collecting 1500 to 1600 ml. of distillate a margin of safety is allowed which should also be sufficient for less volatile propionic acid.

Limits of Applicability. This procedure can be expected to give accurate results on cellulose formate, acetate, acetate propionates, and acetate butyrates of very low butyryl content. It has the particular advantages over the other two that an analysis can be completed in considerably less elapsed time, and the value obtained is a measure of volatile acids. The method thus has some value for control purposes and for obtaining results quickly. The other advantage often permits measurement of the true acetyl value in such compounds as cellulose nitroacetate, cellulose acetate phthalate, and acetates of oxidized or degraded celluloses which themselves consume alkali.

The method gives low results on cellulose butyrates and acetate butyrates containing much butyryl. It fails for esters of other acids higher in the homologous series than butyric, and is also unsatisfactory for mixed esters such as cellulose acetate stearate, which has too high water resistance to be attacked by aqueous acid. In order to be analyzable by this procedure a compound must be in such physical form that it is completely degraded to water-solubility during the analysis.

Precision and Accuracy. Under carefully controlled conditions, precision of ±0.2 per cent acetyl or 0.50 per cent of the numerical value is readily attainable. The accuracy may be poorer, but is within about ±0.3 to 0.5 per cent acetyl (0.8 to 1.3 per cent of the numerical value).

Summary

The methods available for the determination of combined acyl in cellulose esters fall into two classes: saponification with aqueous or alcoholic alkalies or hydrolysis in acid solutions. Difficulties inherent in these methods and of establishing the accuracy of a method are pointed out and discussed.

The modified Eberstadt method is the most accurate and generally satisfactory for the analysis of cellulose acetate and certain other esters of low molecular weight acids. It involves swelling the sample with aqueous alcohol and saponifying with aqueous alkali at room temperature for 1 to 2 days.

A more nearly universal procedure consists of a saponification with 0.25 *N* alcoholic alkali for 16 to 24 hours at not higher than 30° C. However, good accuracy is more difficult to attain by this method than by the Eberstadt method.

The acid distillation method of Ost requires more labor per sample, but is the most rapid of the three methods. It has unique advantages which make it useful for special purposes if not for routine use.

The effects of the most important variables, limits of applicability, precision, and accuracy are given for these three methods.

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Photoelectric Vitamin A Photometer

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WHEN it was established that vitamin A has a peak of absorption at 3280 Å. which has a high degree of persistence in most fish liver oils, it became possible to make vitamin A assays spectrophotometrically (*2*, *3*, *6*). This represented a great saving in time over the animal test. It was soon recognized that an instrument which would measure photoelectrically the absorption of fish liver oils in the neighborhood of 3280 Å. would be a valuable contribution, since, compared with the conventional spectrophotometer, it would offer a means of more rapid and objective vitamin A measurements while also representing a smaller initial cost.

Several such instruments have been constructed, the most prominent of these being the United Drug Company vitamin A meter (made by the G. M. Laboratories, Chicago, Ill., *5*), the Bills and Wallenmeyer electronic photometer (made by the Schoene Equipment Co., Evansville, Ind., *1*), and the photoelectric photometer of Parker and Oser (*7*). The electronic photometer uses, as a light source, an argon lamp, isolating the two argon bands at 3180 and 3380 Å. by means of a Corning No. 986 filter and a nickel chloride solution. The other two instruments use, as a light source, a commercial sodium vapor lamp, isolating the sodium line at 3303 Å. by means of a single Corning No. 986 filter.

In this laboratory, a new photoelectric instrument has been developed for the measurement of vitamin A, using a zinc vapor lamp as light source and a single sodium photocell. The zinc arc spectrum contains six strong lines between 3282 and 3346 Å. When used with a sodium photocell, a single Corning No. 597 filter effectively isolates these six lines, since the sodium photocell does not respond to the zinc arc lines above 5000 Å. which are transmitted by the filter. The transmission of this filter for the isolated group of six lines is about 50 per cent.

This group of zinc lines has certain advantages over the sodium and argon lamps as light sources. As compared with the commercially available sodium lamps, these six zinc lines contain a much higher percentage of the total radiation of the zinc spectrum than the percentage of the total sodium radiation contained in the single sodium line at 3303 Å. This larger amount of available energy results in a simplification of the amplification problem.

Of the three light sources mentioned, the sodium lamp would appear to be superior from the point of view of wavelength characteristics, since its effective radiation is much closer to the peak of vitamin A absorption. That this is not necessarily true is brought out in the following discussion.

When one is dealing with a fish liver oil or concentrate which exhibits a true vitamin A absorption curve, either type of radiation would yield the same result, provided the proper calibration is applied to each. In the case of materials which show a departure from the true vitamin A curve, different results may be obtained, depending upon the type of radiation used. In the absorption spectra of fish liver oils and concentrates, the departures from the true vitamin A curve which are commonly encountered may be divided into two classes. Those which are due simply to interfering absorption, which we may call Type I, are characterized by a decrease in persistence of the peak at 3280 Å. Other departures, which we may call Type II, show a deformation of the vitamin A absorption curve itself and are characterized by a series of absorption peaks on the long wave-length side of 3280 Å., such as reported by Edisbury *et al.* (*4*). In general practice, departures of Type I are encountered much more frequently than those of Type II.

In the case of Type I departures, the correct result—i. e., the result which would be obtained if the interfering absorption were removed—is equal to or less than the lowest of the several results with each of the light sources, since the effect of interfering absorption is always additive. In the study of a large number of absorption curves of Type I, it has been noticed that the departures from the true vitamin A curve are such that the zinc lamp will never yield a higher result than either the sodium or argon lamp. Furthermore, when there is great loss of persistence of the vitamin A peak, the result given by the sodium lamp will be somewhat higher than

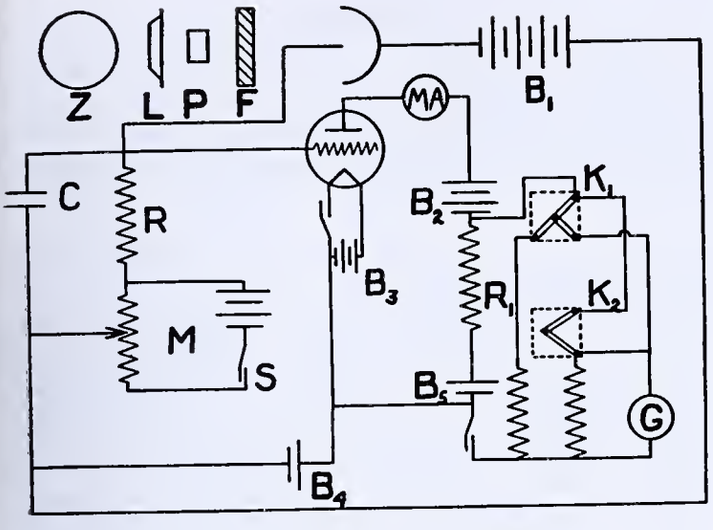

FIGURE 1. DIAGRAM OF PHOTOMETER

B_1. 180 volts
B_2. B-battery, 22.5 volts
B_3. A-battery, 2 volts
B_4. C-battery, 1.5 volts
B_5. 1.5 volts
G. Balanced galvanometer, Leeds & Northrup No. 2320
K_1. Coarse adjustment key
K_2. Fine adjustment key
R. 200 megohms

that of the zinc lamp, while the result given by the argon lamp will be considerably higher than that of the sodium lamp. From this we can assert that, for oils and concentrates showing departures of Type I, the zinc lamp is superior to both the sodium and argon lamps.

In the case of Type II departures, the zinc lamp will usually yield a slightly higher result than either of the other light sources, the sodium lamp being more nearly correct. However, bearing in mind that departures of Type I are more commonly encountered than those of Type II, the zinc lamp is a more satisfactory light source than the argon lamp for the routine vitamin A measurement of fish liver oils and concentrates, and appears to be at least as satisfactory as the sodium lamp.

Details of Instrument

Figure 1 is a diagrammatic representation of the new instrument. Z, the zinc vapor lamp, is manufactured by the Osram Lamp Company of Germany. The lamp, in series with a choke coil, operates on 230-volt alternating current through a constant-voltage transformer (Sola Electric Company). It consumes about 50 watts at 4 amperes. L is a shutter located about 10 cm. from Z and immediately in front of the 1-cm. Uviol glass absorption cell, P. F is a Corning No. 597 filter, 5 mm. thick. The photocell, located immediately behind the filter, is a sodium vacuum photocell manufactured by R. C. Burt of Pasadena, Calif. The amplifying tube shown is a 1B4P used as a triode, with the screen as plate, and has the following characteristics: plate current, 100 microamperes; grid current, 5×10^{-11} ampere; transconductance, 200 micromhos. The current sensitivity of the galvanometer is 1 microampere per mm. R_1, the balancing resistor, is made up of three small radio potentiometers of 20,000, 1000, and 100 ohms in series. The internal resistance drop across R is balanced by M, a Leeds & Northrup student's potentiometer with 12 volts' working voltage.

Switch S is connected by a cord to shutter L, so that opening S closes the shutter and closing S opens the shutter. C is a 0.005-mf. condenser which by-passes the alternating current components of the photocell current, owing to the modulation of the light source in the frequency of the power supply. The 200-megohm resistor, the photocell, the amplifying tube, and the condenser are enclosed in a shielded earthed box.

Operation

With the lamp lit and switch S open, the galvanometer is brought to zero by adjusting resistor R_1. S is then closed, which opens shutter L, and M is adjusted until the galvanometer again reads zero. Again opening S, the galvanometer reading is checked. If the galvanometer does not return to zero, because of drift of the plate current, the operation can be quickly repeated, since the potentiometer is now very near the correct setting. In practice, very little difficulty was encountered due to drift of the plate current.

The photocell dark current has no effect on the potentiometer setting. If I is the first potentiometer setting made with the cell containing a solution of vitamin A oil in 99 per cent isopropanol in the light path, and I_0 is the second potentiometer setting made with the cell removed, the density of the cell plus the solution is given by $\log I_0/I$. The extinction of the dissolved oil is given by subtracting from this the density of the cell containing pure solvent, which has been previously determined by an average of several measurements. This procedure, instead of the usual practice of refilling the cell with pure solvent, shortens the time interval between the two potentiometer settings, which is an obvious advantage. The E value of the oil is obtained in the usual manner by dividing the extinction by the concentration.

Precision

Although the single-photocell type of instrument is admittedly simpler in construction than the double-photocell type, it has the inherent weakness that the results obtained in its use are affected by variations in the intensity of the light source. However, the error so introduced can almost always be made negligibly small by a restriction of the operating density range.

Consider an instrument of the type shown in Figure 1, assuming that the response of the photocell is constant.

Assuming for the moment that the intensity of the light source is constant, I and I_0 will both be subject to a constant uncertainty, ΔI, inherent in the current measuring system. The effect of the variation in the intensity of the light source may be properly introduced by considering I_0 subject to an additional uncertainty, ΔI_0, which represents the variation in the intensity of the light source during the time between the measurement of I and I_0. The resulting fractional uncertainties in $\log \frac{I_0}{I}$ will be as follows, grouping together the terms containing ΔI:

$$e_1 = \frac{\partial\left(\log \frac{I_0}{I}\right)}{\partial I} \times \frac{\Delta I}{\log \frac{I_0}{I}} + \frac{\partial\left(\log \frac{I_0}{I}\right)}{\partial I_0} \times \frac{\Delta I}{\log \frac{I_0}{I}} = \frac{\Delta I}{2.30\, I_0 \log \frac{I_0}{I}}\left(\frac{I_0}{I} + 1\right) \quad (1)$$

$$e_2 = \frac{\partial\left(\log \frac{I_0}{I}\right)}{\partial I_0} \times \frac{\Delta I_0}{\log \frac{I_0}{I}} = \frac{\Delta I_0}{2.30\, I_0 \log \frac{I_0}{I}} \quad (2)$$

e_1 represents the uncertainty introduced by the current measuring system. e_2 represents the uncertainty introduced by the variations in the light source. e_2 and the second term of e_1 are both inversely proportional to $\log \frac{I_0}{I}$.

The first term of e_1 has a minimum value at the point

$$\frac{\partial\left(\frac{\Delta I}{2.30 \log \frac{I_0}{I} \times I}\right)}{\partial I} = 0$$

which gives $\log \frac{I_0}{I} = 0.435$. It increases as we go from this point in either direction.

By assigning values of ΔI and ΔI_0 appropriate to our instrument, we calculate by means of Equations 1 and 2 the fractional variation to be expected in $\log \frac{I_0}{I}$ at different values of the latter.

The uncertainty in the slide wire of the potentiometer is given by the makers as 0.5 millivolt, which is equivalent to 0.000357 I_0. The uncertainty introduced by the reading of the galva-

Table I. Variations in Log $\frac{I_0}{I}$

$\log \frac{I_0}{I}$	e_1	e_2	$e_1 + e_2$
0.1	0.0047	0.0174	0.0221
0.2	0.0027	0.0087	0.0114
0.4	0.0018	0.0044	0.0062
0.8	0.0019	0.0022	0.0041
1.2	0.0029	0.0014	0.0043
1.5	0.0045	0.0012	0.0057

density, the individual determinations were made at approximately the same potentiometer setting, so that the maximum uncertainty in the potentiometer slide wire would probably not be operative. Accordingly, one might expect the variations to be not much greater than e_2, which is indeed true.

In all the other sets of determinations at intermediate densities the variation is within the predicted value.

Table II. Measurements of U. S. P. Reference Oil

Date	6/12/40	6/24/40	6/21/40	6/21/40	6/25/40	6/24/40	6/20/40	6/25/40	6/17/40	6/24/40	6/25/40	6/7/40
Concentration, %	0.1392	0.1315	0.270	0.398	0.5203	0.526	0.673	0.796	0.8835	0.977	0.9825	0.982
$E^{1\%}_{1\ cm.}$	1.507	1.491	1.503	1.510	1.495	1.500	1.495	1.478	1.486	1.457	1.458	1.483
	1.493	1.491	1.506	1.505	1.498	1.502	1.492	1.483	1.489	1.456	1.455	1.485
	1.486	1.491	1.506	1.510	1.492	1.500	1.494	1.475	1.489	1.456	1.456	1.485
	1.501	...	1.514	1.497	...	1.498	1.492	...	1.489	1.461	...	1.486
	1.473	...	1.506	1.508	...	1.495	1.495	...	...	1.458	...	1.485
	1.507	...	1.503	1.505	...	1.495	1.491	...	...	1.455	...	1.487
	1.486	...	1.503	1.505	...	1.495	1.495	...	...	...	...	1.485
	1.493	...	1.499	1.508	...	1.498	1.492	...	...	...	...	1.487
	1.493	...	...	...	...	...	...	...	...	...	...	1.483
	1.493	...	...	...	...	...	...	...	...	...	...	1.487
Av.	1.493	1.491	1.505	1.506	1.495	1.498	1.493	1.479	1.488	1.457	1.456	1.485
Av. extinction	0.208	0.196	0.406	0.600	0.779	0.788	1.005	1.177	1.312	1.423	1.430	1.459

nometer and the drift of the plate current will be taken to be 0.3 galvanometer division, equivalent to 0.000125 I_0.

Summing up, we have $\Delta I = 0.00476\ I_0$.

In order to estimate ΔI_0, the output of the lamp was measured once a minute for a continuous period of 1 hour. The maximum change during any 1-minute interval was 0.4 per cent. Since the actual lapse of time between the measurement of I and I_0 is about 1 minute, we take $\Delta I_0 = 0.004\ I_0$.

With these values, Table I of fractional variations in log I_0/I at different values was calculated. From Table I we see that if e_2 were zero we would have a theoretical precision of about ±0.25 per cent in the density range 0.2 to 1.2. With e_2 as calculated we have, from the last column, a precision of about ±0.5 per cent in the density range 0.45 to 1.5. This is better precision than is generally regarded as necessary for vitamin A work. With a variation in the light source three times that which we have, and in the density range 0.45 to 1.5, the theoretical precision would be about ±1.5 per cent, a tolerable figure for this type of work. This justifies the statement made above that, by a proper restriction of the working density scale, we may get results of good precision on a single photocell instrument in spite of sizable variations in the intensity of the light source.

The operating range 0.2 to 1.5, which gives an over-all theoretical precision of ±1 per cent, was selected.

Measurements of U. S. P. Reference Oil

A series of 74 determinations was made on U. S. P. reference oil dissolved in 99 per cent isopropanol, covering an extinction range of 0.2 to 1.5 and extending over a period of 18 days. The results are given in Table II.

Ten determinations were made at an extinction of 0.208 on June 12. The extreme variation of these determinations is ±1.1 per cent, while the theoretical precision at that point, from Table I, is also equal to ±1.1 per cent. Three determinations made on the following day at approximately the same extinction also fell within these limits.

Nine determinations made on June 24 and 25 at an extinction of 1.43 show a variation of ±0.20 per cent and ten results on June 7 show a variation of ±0.14 per cent.

These results, at the two extremes of the density range, are of particular interest because at the extreme low density e_2 dominates e_1 by about 3 to 1, whereas at the extreme upper density e_1 dominates e_2 by about 4 to 1. At the lower density range the agreement with prediction is extremely good. At the extreme upper range the variation is much less than the calculated value. This may be readily explained as follows: In each of the two sets of determinations made at the high

Although the reproducibility shown in each set of determinations is within the theoretical value, surprisingly large variations are evident between the averages of the different sets made on different days at approximately the same extinction—for example, although the determinations made at the very low extinction on June 12 and 24 are in very good agreement, the averages of determinations made at the very high density on June 7 and 24–25 differ by 2 per cent. This variation is inconsistent with the agreement within the individual groups. A variation in the response of the photocell at different times would seem to be the only logical explanation. Such a variation would be more noticeable the higher the density. In view of this it was decided to restrict the operating density range to 0.2 to 1.2, which is much greater than the operating range of the conventional spectrophotometer. The complete variation of all the determinations on U. S. P. reference oil within this density range is ±1.4 per cent, which compares well with the theoretical value of ±1.1 per cent.

The average E value of the U. S. P. reference oil within the selected density range was 1.495. With the Bausch & Lomb spectrophotometer the author has obtained on U. S. P. reference oil an average E value of 1.555 on the oil and 1.490 on its unsaponifiable, and is using a conversion factor of 2000 for this instrument. In order to render results obtained on the new instrument comparable with those obtained on the spectrophotometer, he adopted the factor 2080 for the former. Using this conversion factor, determinations were made on 25 miscellaneous samples of fish liver oils and concentrates ranging in potency from 1130 to 326,000 units of vitamin A per gram, both on the new instrument and on the Bausch & Lomb spectrophotometer. The maximum difference between the two instruments on any of these samples was 3 per cent. This is consistent with the accuracy of ±1.4 per cent for the new instrument and ±1.5 per cent for the spectrophotometer, which is the precision assigned to this instrument as the result of a large number of determinations.

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Precipitation of Zinc Sulfide from a Solution of Ammonium Citrate and Citric Acid

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THE Waring method for the separation of zinc, published in 1907 (*11*), was developed as the result of an extensive study of the analyses of zinc ores, conducted by the Committee on Uniformity in Technical Analysis of the AMERICAN CHEMICAL SOCIETY (*2*). This latter report indicated that the precipitation of zinc as zinc sulfide was the only satisfactory method available for the final separation of zinc. This procedure has been studied extensively, so that our knowledge of the conditions under which zinc sulfide may be precipitated quantitatively is as complete as it is for any analytical method available to chemists.

TABLE I. PRECIPITATION OF ZINC SULFIDE FROM A SOLUTION OF AMMONIUM CITRATE AND CITRIC ACID

(0.4259 gram of $ZnSO_4$ taken)

Excess of 1 *M* Citric Acid Used	$Zn_2P_2O_7$ Found	$ZnSO_4$ Calcd.	Deviation from Mean
Ml.	*Gram*	*Gram*	*Mg.*
15	0.4020	0.4259	0.0
15	0.4019	0.4258	−0.1
20	0.4024	0.4263	+0.4
20	0.4025	0.4265	+0.6
25	0.4016	0.4253	−0.6
25	0.4018	0.4256	−0.3
25	0.4017	0.4255	−0.4
25	0.4025	0.4265	+0.6
30	0.4020	0.4259	0.0
30	0.4018	0.4256	−0.3
40	0.4021	0.4260	+0.1
40	0.4026	0.4265	+0.6
40	0.4023	0.4262	+0.3
40	0.4023	0.4262	+0.3
50	0.4009	0.4247	−1.2
50	0.4012	0.4250	−0.9
50	0.4023	0.4262	+0.3
50	0.4023	0.4262	+0.3
	Av.	0.4259	

The Waring method was studied critically, and was modified by Fales and Ware in 1919 (*4*), with the result that the present Waring-Fales-Ware method for the separation of zinc is an elegant procedure, and the one most generally employed today. Long experience with the separation of zinc as zinc sulfide has shown that the quantitative precipitation (for analytical purposes) must be effected from an acid solution, pH 2 to 3, and that any buffer solution which will maintain the reaction within this range is satisfactory. Weiss (*12*) precipitated zinc sulfide merely from a 0.01 *N* solution of sulfuric acid but Lundell, Hoffman, and Bright (*8*) and Jeffries and Swift (*6*) suggested a "sulfate-hydrosulfate" buffer. Mayr (*9*) regulated the acidity with a solution of chloroacetic acid and sodium acetate.

The Waring method as modified by Fales and Ware employs formic acid and ammonium formate as a buffer, and in addition they add citric acid to form a "citrate complex" with iron. Citric acid is of moderate strength, PKa_1 −3.06, PKa_2 −4.74, and PKa_3 −5.40, somewhat stronger than formic acid, and therefore the statement of Smith (*10*) that "a mixture of citric acid and ammonia in suitable proportions would serve not only to keep iron in solution by complex formation, but also as a buffer" would seem to be reasonable. The investigation upon which this paper is based was undertaken in order to test the validity of the above statement.

The experimental results presented herewith prove conclusively that the T. B. Smith statement is entirely justified. Accordingly, the Waring-Fales-Ware method may be simplified by eliminating the use of formic acid and ammonium formate.

Experimental

The studies reported here have been planned to parallel as closely as possible the work of Fales and Ware, although pH determinations have not been made. Zinc solutions used were standardized by the Waring-Fales-Ware method, but zinc was determined by weighing as zinc pyrophosphate (*5*). This method of determination was also employed in all the studies, in order to ensure comparative results. Series of experiments were conducted, following the technique of Fales and Ware, but the solutions were buffered with ammonium citrate and citric acid, instead of with solutions of formic acid and ammonium formate.

STANDARD SOLUTIONS. A solution of zinc sulfate was prepared by dissolving 20 grams of pure zinc oxide in dilute sulfuric acid. This was neutralized with ammonium hydroxide, made slightly acid with sulfuric acid, and was then diluted to approximately 2.5 liters. The solutions were standardized as stated above, making at least six determinations. In the standardization of one solution eight results were obtained; 25-ml. aliquots were taken and the zinc was weighed as zinc pyrophosphate, and these results were calculated to zinc sulfate: 0.4261, 0.4255, 0.4256, 0.4259, 0.4261, 0.4258, 0.4257, 0.4264 gram of zinc sulfate; mean, 0.4259 gram of zinc sulfate, standard deviation from the arithmetic mean of a single determination, 0.3 mg. (7 parts in 10,000); standard deviation of the arithmetic mean, 0.1 mg. (2.3 parts in 10,000).

The solutions of interfering elements were not standardized, but were prepared by dissolving weighed quantities of the pure substances, ferrous sulfate, manganous sulfate, aluminum sulfate, nickel sulfate, and cobalt oxide, and diluting to definite volumes. These solutions were prepared so that 20 ml. of the iron solution contained 0.19 gram of iron and the others contained 0.17 gram of the element.

REAGENTS. Formic mixture was prepared according to the directions of Fales and Kenney (*3*). Citric acid was 1 *M* (200 grams per liter). Ammonium sulfate, 200 grams per liter; ammonium thiocyanate, 200 grams per liter.

TABLE II. SEPARATION OF ZINC FROM IRON

Expt.	Fe Taken	$Zn_2P_2O_7$ Found	$ZnSO_4$ Calcd.	Difference
	Gram	*Gram*	*Gram*	*Mg.*
Excess 1 *M* citric acid, 25 ml.; 0.4766 gram of $ZnSO_4$ taken				
1	0.049	0.4501	0.4768	+0.2
2	0.049	0.4494	0.4761	−0.5
3	0.098	0.4517	0.4785	+1.9
4	0.098	0.4514	0.4782	+1.6
9	0.147	0.4517	0.4785	+1.9
10	0.147	0.4512	0.4780	+1.4
Excess 1 *M* citric acid, 40 ml.; 0.4259 gram of $ZnSO_4$ taken				
5	0.098	0.4008	0.4246	−1.3
6	0.098	0.3562	0.3773	Result discarded
7	0.098	0.4025	0.4264	+0.5
8	0.098	0.4026	0.4265	+0.6
13	0.196	0.4026	0.4265	+0.6
14	0.196	0.4026	0.4265	+0.6
17	0.343	0.4039	0.4279	+2.0
18	0.343	0.4025	0.4264	+0.5
Excess 1 *M* citric acid, 50 ml.; 0.4766 gram of $ZnSO_4$ taken for Nos. 11 and 12, 0.4259 gram of $ZnSO_4$ for Nos. 15 and 16				
11	0.196	0.4510	0.4778	+1.2
12	0.196	0.4499	0.4766	0.0
15	0.343	0.4036	0.4275	+1.6
16	0.343	0.4040	0.4280	+2.1

TABLE III. SEPARATION OF ZINC FROM IRON BY WARING-FALES-WARE METHOD

(Determinations run in conjunction with Nos. 11, 12, 15, and 16 in Table II)

$ZnSO_4$ Taken	Fe Taken	$Zn_2P_2O_7$ Found	$ZnSO_4$ Calcd.	Difference
Gram	*Gram*	*Gram*	*Gram*	*Mg.*
0.4766	0.196	0.4501	0.4765	+0.2
0.4766	0.196	0.4506	0.4773	+0.7
0.4259	0.343	0.4040	0.4280	+2.1
0.4259	0.343	0.4032	0.4271	+1.2

TABLE IV. SEPARATION OF ZINC FROM MANGANESE, ALUMINUM, AND NICKEL

(Excess 1 *M* citric acid, 50 ml.)

Element Added, 0.17 Gram	$ZnSO_4$ Taken	$Zn_2P_2O_7$ Found	$ZnSO_4$ Calcd.	Difference
	Gram	*Gram*	*Gram*	*Mg.*
Manganese	0.4259	0.4022	0.4261	+0.2
	0.4259	0.4018	0.4256	−0.3
	0.4090	0.3861	0.4090	0.0
	0.4090	0.3858	0.4087	−0.3
Aluminum	0.4259	0.4024	0.4263	+0.4
	0.4259	0.4022	0.4261	+0.2
	0.4090	0.3863	0.4092	+0.2
	0.4090	0.3864	0.4093	+0.3
Nickel	0.4259	0.4019	0.4257	−0.2
	0.4259	0.4016	0.4254	−0.5
	0.4090	0.3865	0.4094	+0.4
	0.4090	0.3859	0.4088	−0.2

BUFFER SOLUTIONS. The standard solution of zinc sulfate (25 ml.) was transferred to a 500-ml. Erlenmeyer flask and 6 *M* ammonium hydroxide was added until a slight permanent precipitate of zinc hydroxide formed. Citric acid (25 ml. of 1 *M* solution) was added and neutralized with ammonium hydroxide, using methyl orange indicator, and this was followed by 25 ml. of the solution of ammonium sulfate. The desired volume of citric acid was now added, and the total volume was adjusted to 200 ml. In experiments conducted with solutions of zinc sulfate only, the precipitate was allowed to stand over-night before filtering, but when interfering ions were present the precipitate was separated by filtration as soon as the supernatant liquid was clear, usually within one hour.

OUTLINE OF EXPERIMENTS. The first series of experiments was conducted in order to establish the optimum proportions of citric acid and ammonium citrate necessary for the precipitation of zinc sulfide. After the neutralization of the 25 ml. of 1 *M* citric acid, 15-, 20-, 25-, 30-, 40-, and 50-ml. portions of 1 *M* citric acid in excess were added, and zinc sulfide was precipitated. The results of these experiments are tabulated in Table I.

A detailed study of the separation of zinc from iron was then made in the second series of experiments (Table II). Table III presents some results of the separation of zinc from iron by the standard Waring-Fales-Ware procedure.

The third series of experiments shows the application of the new technique to the separation of zinc from manganese, aluminum, and nickel (Table IV).

The separation of zinc from cobalt was studied in considerable detail. Ammonium thiocyanate was used instead of ammonium sulfate for salting out zinc sulfide, and in addition, for minimizing the postprecipitation of cobalt sulfide by forming a thiocyano complex ion with cobalt. The results of these experiments are presented in Tables V and VI.

In Table VII is recorded a series of experiments planned to show whether or not zinc sulfide can be dried on a Gooch crucible and weighed as such. The results reported clearly demonstrate that this procedure is not reliable.

In the final series of experiments, the new method is applied to the determination of zinc in three zinc ores, National Bureau of Standards Samples 2A and 113, and a Polytechnic Institute student sample. The last sample was analyzed by a sophomore student in the senior author's class in quantitative analysis. The results of these experiments are tabulated in Table VIII.

Discussion of Results

AMMONIUM CITRATE AND CITRIC ACID AS BUFFER. Of the eighteen results reported in Table I, all are apparently within the limit of the experimental error of the Waring-Fales-Ware method. The arithmetic mean of the eighteen determinations is 0.4259 gram of zinc sulfate, which is the same as that found in the eight determinations carried out in standardizing the solution by the Waring-Fales-Ware procedure. The standard deviation of a single determination from the arithmetic mean of these eighteen determinations is 0.5 mg. or 12 parts in 10,000. The standard deviation of the arithmetic mean is 0.12 mg. or 2.8 parts in 10,000. When we compare these deviations with those of the determinations made in standardizing the solution, there is no significant difference in the results obtained by the Waring-Fales-Ware method and those obtained by using the citrate–citric acid buffer. Treatment of these data by analysis of variance also shows that the two methods do not give results which are significantly different.

It is also evident that there is considerable latitude in the amount of excess citric acid which may be added. Indeed, in this series the amount of excess citric acid does not have a significant effect upon the results. This conclusion is proved by analysis of variance in this series. We are therefore justified in concluding that ammonium citrate and citric acid used serve together as a satisfactory buffer for regulating the acidity of solutions from which zinc sulfide is precipitated quantitatively.

TABLE V. SEPARATION OF ZINC FROM COBALT

(0.17 gram of Co taken)

20% Solution of NH_4SCN	$Zn_2P_2O_7$ Found	$ZnSO_4$ Calcd.	Difference
Ml.	*Gram*	*Gram*	*Mg.*
Excess 1 *M* citric acid, 50 ml.; 0.4259 gram of $ZnSO_4$ taken			
[a]	0.4127	0.4372	+11.3
[a]	0.4136	0.4381	+12.2
25	0.4037	0.4315	+ 5.6
25	0.4088	0.4331	+ 7.2
50	0.4031	0.4270	+ 1.1
50	0.4026	0.4265	+ 0.6
75	0.3893	0.4124	−13.5
75	0.3870	0.4100	−15.9
50	0.4014	0.4252[b]	−0.7
50	0.4009	0.4247[b]	−1.2
Excess 1 *M* citric acid, 40 ml.; 0.4782 gram of $ZnSO_4$ taken			
50	0.4522	0.4790	+0.8
50	0.4509	0.4777	−0.5
50	0.4552	0.4822	Results discarded
50	0.4557	0.4721	Results discarded
50	0.4503	0.4770[b]	−1.2
50	0.4504	0.4771[b]	−1.1
Excess 1 *M* citric acid, 50 ml.; 0.4782 gram of $ZnSO_4$ taken of 1st two experiments, 0.4090 gram of $ZnSO_4$ taken for other experiments			
50	0.4519	0.4787	+0.5
50	0.4507	0.4775	−0.7
50	0.3890	0.4120	+3.0
50	0.3907	0.4139	+4.5
50	0.3896	0.4127	+3.7
50	0.3905	0.4137	+4.7
50	0.3892	0.4123	+3.3
50	0.3880	0.4110	+2.0
50	0.3861	0.4090[b]	0.0
50	0.3859	0.4088[b]	−0.2

[a] 25 ml. of ammonium sulfate employed instead of ammonium thiocyanate.
[b] No cobalt present.

SEPARATION OF ZINC FROM IRON. The second series of experiments consists of a detailed study of the separation of zinc from iron. The citrate–citric acid solution not only serves to regulate the acidity, so that the solubility product of ferrous sulfide will not be exceeded, but also reduces the concentration of the ferrous ion by forming a ferrous citrate complex ion or molecule. Table II indicates that there is

some contamination of zinc sulfide with iron, and this increases with the concentration of iron in the solution. If we consider the last two sets of results, the standard deviation of a single determination from the results according to the Waring-Fales-Ware standardization is 1.0 mg. or approximately 2.5 parts per 1000. These results are high. Table III reports four results obtained by following the Waring-Fales-Ware method. These results deviate from the standardization values in the same way and the order of magnitude is the same. The standard deviation is 1.25 mg. for a single determination. Fales and Ware (*4*) found that some iron contaminated the precipitate of zinc sulfide if iron were present in the solution from which the separation had been effected. It is concluded, therefore, that the new technique is just as effective as the Waring-Fales-Ware technique in separating zinc from iron.

Separation of Zinc from Manganese, Aluminum, and Nickel. The separation of zinc from manganese, from aluminum, and from nickel is surprisingly satisfactory. Of the twelve results reported in Table IV, only one deviates by more than one part in 1000. We may conclude, therefore, that these separations are as nearly perfect as the techniques warrant.

Separation of Zinc from Cobalt. A completely satisfactory method for the separation of zinc from large amounts of cobalt has not been developed. Apparently the contamination of zinc sulfide with cobalt sulfide is due to postprecipitation similar to the contamination of copper sulfide with zinc sulfide (*7*). Caldwell and Moyer (*1*) employed acrolein for minimizing the postprecipitation of cobalt sulfide. A study is made in the series of experiments reported in Table V of the use of ammonium thiocyanate as a reagent to minimize the postprecipitation of cobalt sulfide. Ammonium thiocyanate acts also to salt out zinc sulfide. Using a 20 per cent solution of ammonium thiocyanate, the contamination with cobalt sulfide is reduced considerably. It appears that 50 ml. of the 20 per cent solution of ammonium thiocyanate are as much as can be used safely. However, in all cases, cobalt sulfide can be seen as a contaminant, and it appears after zinc is almost completely precipitated. In the six determinations (bracketed) toward the bottom of the table, we find a mean value of 0.4126 gram of zinc sulfate, or a constant error of 3.4 mg. (9 parts in 1000). The standard deviation from the arithmetic mean of a single observation is 0.95 mg. (2.3 parts in 1000), and the standard deviation of the arithmetic mean is 0.4 mg. (1 part in 1000).

Certain determinations reported here appear satisfactory, and it was thought that the time of precipitation was an important factor in the amount of cobalt sulfide present in the precipitate. Table VI demonstrates conclusively that this postulation is valid. Thiourea and thiophenol do not prevent the postprecipitation of cobalt sulfide. Further studies to establish optimum conditions are projected.

Table VI. Effect of Time of Precipitation on Contamination of Zinc Sulfide with Cobalt Sulfide

(20% solution of NH_4SCN taken, 50 ml.; 0.17 gram of Co taken)

$ZnSO_4$ Taken	Time of Pptn.	$Zn_2P_2O_7$ Found	$ZnSO_4$ Calcd.	Difference
Gram	*Min.*	*Gram*	*Gram*	*Mg.*
0.4095	20	0.3906	0.4138	+4.3
0.4095	20	0.3907	0.4139	+4.4
0.4095	45	0.3924	0.4157	+6.2
0.4095	45	0.3933	0.4166	+7.1

Table VII. Weighing Zinc as Zinc Sulfide

$ZnSO_4$ Found: Waring-Fales-Ware method	$ZnSO_4$ Found: Citric acid method	ZnS Found	ZnS Calcd. as $ZnSO_4$	ZnS, Converted to $Zn_2P_2O_7$, Calcd. as $ZnSO_4$
Gram	*Gram*	*Gram*	*Gram*	*Gram*
0.4242	0.4249	0.2685	0.4449	0.4249
0.4249	0.4243	0.2681	0.4442	....
0.4238	....	0.2670	0.4423	0.4242
0.4246	....	0.2665	0.4415	....
Av. 0.4244	0.4246			

Table VIII. Analysis of Zinc Ores by Use of Citric Acid–Citrate Buffer

Sample	Zn Found	Deviation from Mean	Deviation from Reported Result
	%	%	%
U. S. B. S. 2A	30.41	−0.03	−0.12
	30.41	−0.03	−0.12
	30.47	+0.02	−0.06
	30.46	−0.02	−0.07
U. S. B. S. 113	60.98	+0.04	−0.12
	60.89	−0.05	−0.21
Student sample	19.19[a]	−0.04	[b]
	18.48[a]	Result rejected	
	19.23[a]	0.00	
	19.28[a]	+0.05	

[a] Analyses made by Aaron Wexler, Polytechnic '42, sophomore in course in quantitative analysis.
[b] Value used in checking students' results, 19.17.

Weighing Zinc as Zinc Sulfide. The possibility of filtering zinc sulfide on a tared Gooch crucible, drying at 110° C., and weighing directly, was tested in the experiments summarized in Table VII. Zinc sulfide was precipitated from solutions of zinc sulfate by the Waring-Fales-Ware method and by the ammonium citrate–citric acid procedure, the zinc was weighed as zinc pyrophosphate, and the results were calculated as zinc sulfate (columns 1 and 2, Table VII). Zinc sulfide which had been precipitated by the Waring-Fales-Ware method was filtered onto Gooch crucibles and weighed. The results are given in column 3, and in column 4 the zinc sulfide is calculated to weight of zinc sulfate. In column 5 are given results in terms of zinc sulfate of two precipitates of zinc sulfide (first and third items in column 3) which were converted to zinc pyrophosphate. We conclude, therefore, that it is not permissible to weigh zinc as zinc sulfide by the technique described here.

Analysis of Zinc Ores

Samples of three zinc ores were analyzed by the procedure developed as a result of these studies.

The zinc ores are dissolved and silicic acid, copper, cadmium, and other metals of the hydrogen sulfide group are removed in the usual way. The solution of zinc is placed in a 500-ml. or 750-ml. Erlenmeyer flask and 6 *M* ammonium hydroxide is added until the precipitate of zinc hydroxide just fails to dissolve. Citric acid (25 ml. of 1 *M* solution) is added and made neutral to methyl orange by adding 6 *M* ammonium hydroxide. An excess of citric acid (50 ml. of 1 *M* solution) is now added and also 25 ml. of a 20 per cent solution of ammonium sulfate (50 ml. of a 20 per cent solution of ammonium thiocyanate are used instead of ammonium sulfate if cobalt is present). The volume is adjusted to 200 ml., the solution is heated to 60°, and air is replaced with hydrogen sulfide. The heating of the solution is continued until a temperature of 95° is attained, and the exit tube is closed. The solution is allowed to cool and become saturated with hydrogen sulfide under the pressure of the hydrogen sulfide from the generator, and is agitated frequently during a period of 20 to 40 minutes. The precipitate is allowed to settle, separated by filtration, and washed with a 0.1 *M* solution of citric acid saturated with hydrogen sulfide. Zinc may now be determined by any of the standard methods. In these analyses the zinc is weighed as zinc pyrophosphate (*5*).

An examination of the results of the analyses as recorded in Table VIII shows that the method described above is very satisfactory.

Conclusion

The studies described in this paper demonstrate that in the Waring-Fales-Ware method for the separation of zinc the formate–formic acid buffer is not necessary, since a mixture of

ammonium citrate and citric acid serves not only to keep iron in solution but as an efficient buffer solution; that the results obtained by the use of citric acid–ammonium citrate as a buffer are comparable to those obtained by the Waring-Fales-Ware method and have a precision of 1 part in 1000; that zinc cannot be determined by weighing as zinc sulfide; and that 50 ml. of a 20 per cent solution of ammonium thiocyanate serve to salt out zinc sulfide and to minimize the post-precipitation of cobalt sulfide.

Acknowledgment

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Determination of Thiamin by the Thiochrome Reaction

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BECAUSE of the time and cost involved in the biological assay of thiamin, several microbiological and chemical methods have been proposed for its determination. Of the chemical methods, the thiochrome procedure first proposed by Jansen (*3*) has received a considerable amount of attention. This method is based on the measurement of the fluorescence produced by thiochrome formed by the oxidation of thiamin with potassium ferricyanide in an alkaline solution.

The original method of Jansen has been modified by Karrer and Kubli (*4*), and more recently by Hennessy and Cerecedo (*2*), who introduced the use of a synthetic zeolite, Decalso, for absorbing the thiamin, and thereby separating it from substances interfering with the reaction. They also were the first to employ a sensitive photoelectric instrument (the Pfaltz & Bauer fluorophotometer) for measurement of the fluorescence.

The work described in this paper attempts to define more exactly than has been done previously the optimal conditions for carrying out the thiochrome procedure, as well as to suggest some improvement in the equipment recommended for the determination.

Description of Method

Generally a 3- to 5-gram sample is used for the determination. The sample should be in a finely pulverized state and representative of the material being analyzed. In the case of fresh vegetables, it has been found convenient first to freeze with solid carbon dioxide, then grind in an ordinary meat grinder, and weigh a representative sample of the ground frozen vegetable. Grinding and weighing are conducted in a refrigerated room held below freezing. With materials of high fat content, it may be necessary first to extract a weighed amount of the material with ether in a Soxhlet extractor and then carry out the analysis on the fat-free residue.

The weighed sample is placed in a specially designed extraction tube of about 75-cc. capacity, to which 50 cc. of 0.04 *N* sulfuric acid are added, giving a liquid to solid ratio of about 10 to 1. The pH (1 to 2) of this extraction mixture is optimal for the extraction of the vitamin and sufficiently acid to prevent its destruction.

The extraction assembly is illustrated in Figures 1 and 2. The extraction tube is attached by means of a ground-glass joint, *A*, to a small glass water condenser, *B*, through which extends a glass stirrer, *C*, attached to a small electric motor with a variable rheostat. The heat required for the extraction may be furnished by a microburner, electric hot plate, or hot water bath as shown in Figure 2.

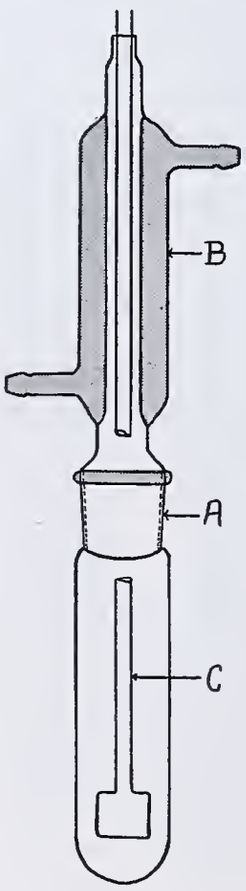

Figure 1

Before any heat is applied the mixture should be thoroughly stirred to prevent charring of the sample. The extraction is carried out at the boiling point of the mixture for approximately one hour with continuous stirring. Although for certain samples a shorter period of extraction may be employed, an hour's extraction will ensure complete solution of the vitamin. At the conclusion of the extraction, each of the stirrers is carefully washed down with 5 cc. of distilled water delivered from a measuring pipet and the tubes are cooled to room temperature by placing them in running water.

As the pH of the solution following the extraction usually lies between 1 and 2, it is necessary to increase the pH to the range of 4 to 5 in order to obtain optimal conditions for the enzymatic hydrolysis of any cocarboxylase present in the extract. Although Hennessy and Cerecedo (*2*) employed 1 *N* sodium hydroxide for this purpose, it was felt that the use of this reagent might lead to a loss of thiamin due to the development of a local area of high alkaline concentration before

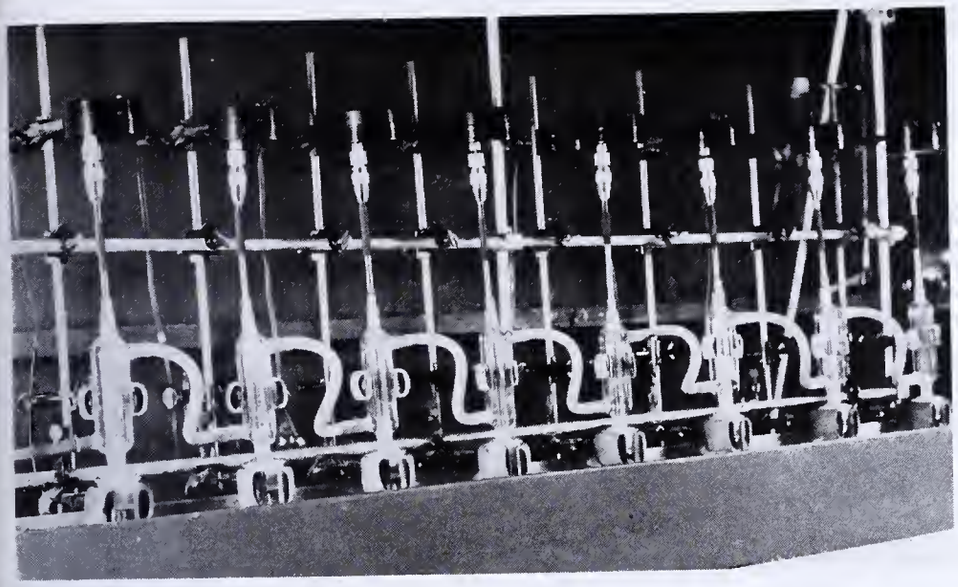

FIGURE 2. EXTRACTION ASSEMBLY

complete mixing could be brought about. Therefore, the enzyme, clarase, used for the hydrolysis, was prepared in a sodium acetate–acetic acid buffer, so that the extraction mixture, after the addition of the clarase solution, yielded the desired pH. The sodium acetate–acetic acid buffer solution (pH 4.5) is prepared by adding 54.40 cc. of glacial acetic acid to 66.9380 grams of anhydrous sodium acetate together with sufficient distilled water to obtain a solution of the reagents, and then transferred to a 1-liter volumetric flask and made up to volume with distilled water.

Although with some cereal products, the pH of the solution during enzymatic hydrolysis may exceed 5 if sodium hydroxide is employed to bring about the desired pH, this was not found to be the case with the buffered mixture. In the present procedure, 10 cc. of a 5 per cent solution of clarase (prepared fresh daily as previously described) are added to each extraction tube. The content of each tube is then thoroughly mixed with a glass stirring rod and the tube, containing the stirring rod, is transferred to an incubation oven maintained at a temperature of 45° C. The tubes are incubated for 2 hours at this temperature with frequent stirring.

Following the incubation period, each stirrer is carefully washed down with 1 cc. of distilled water delivered from a measuring pipet and the extraction tubes are cooled to room temperature. The tubes are then centrifuged at a high speed until a clear supernatant liquid is obtained. An aliquot containing approximately 5 micrograms of thiamin is then pipetted out of the supernatant liquid into a 50-cc. beaker containing 5 cc. of 2 per cent acetic acid.

The acetic acid solution containing the thiamin is heated just to boiling on an electric hot plate and passed through a 5-cm. (2-inch) column of activated (*2*) 60- to 80-mesh Decalso, resting on a plug of spun glass in a specially designed absorption tube. This tube is similar in design to that described in the procedure of Merck & Co. (*7*). Each absorption tube is attached by means of a one-hole rubber stopper to a 125-cc. glass suction flask as shown in Figure 3.

When the vitamin-containing solution reaches the bottom of the Decalso column, a gentle suction is momentarily applied, so that a rate of flow of approximately 1 cc. per minute is obtained. After the vitamin solution has passed through, the beaker and the column are rinsed several times with hot distilled water and the washings are passed through the column. By testing the filtrate and washings for thiamin, this procedure was found to lead to practically 100 per cent absorption of the vitamin on the Decalso column.

The vitamin is eluted from the column by use of 25 cc. of a hot 25 per cent solution of potassium chloride, following the procedure of Hennessy and Cerecedo (*2*). A 25 per cent solution of sodium chloride is equally effective, provided the Decalso column has been previously activated with this reagent instead of potassium chloride. A 5-cc. aliquot of this eluate is then pipetted into a separatory funnel for carrying out the oxidation of the thiamin to the fluorescent material, thiochrome. It has been found preferable to use separatory funnels equipped with No-Lub stopcocks for this purpose rather than those described by Hennessy and Cerecedo (*2*). To this 5-cc. aliquot is added 1 cc. of a freshly prepared solution containing 0.002 gram of potassium ferricyanide and 0.45 gram of sodium hydroxide. Twenty cubic centimeters of isobutyl alcohol are finally added and the separatory funnel is shaken for one minute in a mechanical shaking machine and then centrifuged. The lower aqueous layer is drawn off, the isobutyl alcohol layer is treated with 2 grams of anhydrous sodium sulfate, and the separatory funnel is again centrifuged to eliminate any suspended particles of sodium sulfate. The oxidation of the thiamin and the subsequent extraction of the resulting thiochrome should be carried out as quickly as possible in order to obtain consistent results.

Each batch of isobutyl alcohol should be tested for its fluorescence. If the fluorescence measured in the fluorophotometer is more than that given by an equivalent amount of distilled water, the alcohol should be redistilled from an all-glass apparatus, collecting only that fraction distilling at 106° to 107° C. Fluorescent materials were found to be present in the higher boiling fractions.

Following the sodium sulfate treatment, a suitable aliquot (15 cc.) of the isobutyl alcohol solution containing the thiochrome is pipetted into the cuvette of the Pfaltz & Bauer fluorophotometer and its fluorescence is measured according to the procedure of Hennessy and Cerecedo (*2*). The instrument should be checked before each measurement with a standard quinine solution and the light intensity adjusted to the desired value by changing the setting of the iris diaphragm. The frequent checking of the instrument with quinine sulfate has been found useful, as the light source used in it has only a limited life and the prolonged use of such a bulb will result in variable readings.

It has also been found desirable to run daily a corresponding oxidation of a standard solution (1 cc. = 1 microgram) of pure thiamin chloride and to compare the galvanometer readings of the unknown samples with that of the standard solution, rather than to depend entirely upon a calibration curve for determining the concentration of thiamin in the unknown. The standard solution of thiamin chloride is prepared in 0.5 per cent solution of chlorobutanol at a pH of 3.2 and is kept under refrigeration when

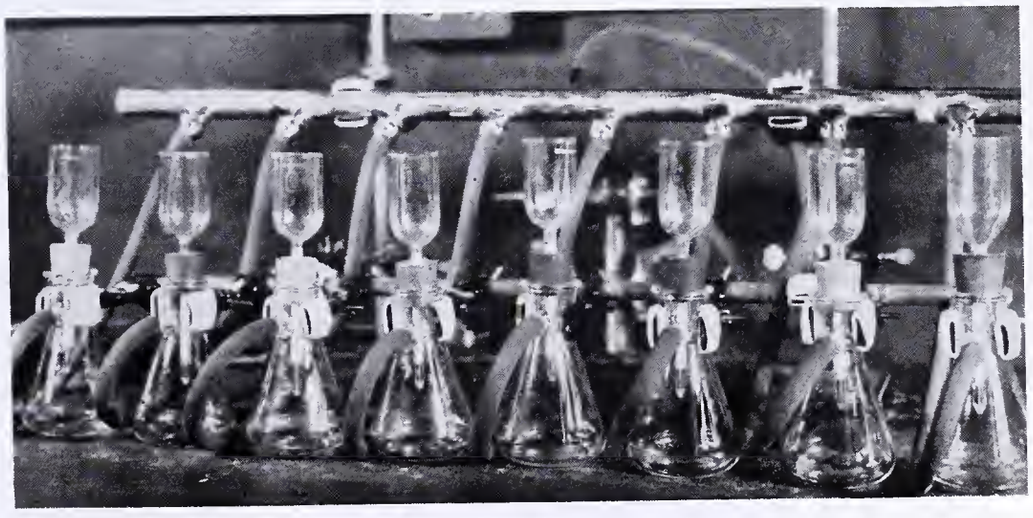

FIGURE 3. SETUP OF ABSORPTION TUBES

not in use. The chlorobutanol prevents mold growth and does not influence the fluorescence measurements.

The cuvette in which the fluorescence measurements are to be made should always be placed in the same position in the fluorophotometer before each measurement, as the walls of the cuvette may vary in their light transmittancy. Cuvettes have also been found to vary among themselves in this respect.

A "complete blank" is run daily for each series of determinations. This consists of 50 cc. of 0.04 *N* sulfuric acid, 10 cc. of 5 per cent clarase solution, and 6 cc. of distilled water. The same incubation and centrifuging procedure is applied to the blank as to the samples. A volume of aliquot is taken from the centrifuged blank corresponding to that used in each determination. Five cubic centimeters of the potassium chloride eluate from the Decalso column, through which the blank solution has been passed, are then oxidized under the same conditions employed for the samples under test, and its fluorescence is determined. The latter is the correction to be applied in arriving at the true thiamin value of each sample. In the case of the standard thiamin solution, only an "oxidation blank" correction need be applied. This is the fluorescence resulting from a mixture of 0.45 gram of sodium hydroxide, 20 cc. of isobutyl alcohol, and 5 cc. of distilled water.

The blanks described do not cover the possibility that some fluorescent material other than thiamin may be eluted from the Decalso. A suggestion that this is not a serious contingency is contained in the comparative chemical and bioassays shown in Table VII.

Critical Study of Steps in Procedure

Enzymatic Hydrolysis. Lohmann and Schuster (*6*) have shown that a great part of the naturally occurring thiamin may be present in the form of its pyrophosphoric acid ester known as cocarboxylase. Although cocarboxylase is converted by alkaline ferricyanide to the pyrophosphoric ester of thiochrome, Kinnersley and Peters (*5*) have shown that this compound cannot be extracted from an aqueous solution with isobutyl alcohol. Therefore, it is necessary to hydrolyze the pyrophosphoric acid group from the cocarboxylase prior to oxidizing it with the alkaline ferricyanide. Hennessy and Cerecedo (*2*) employed an enzyme preparation prepared from beef kidney for this purpose. As the preparation of such material is tedious and time-consuming, the adaptability of other enzyme products was investigated. The use of takadiastase has already been mentioned (*7*). Clarase, mylase, and a mylase with high phosphatase activity were also studied.

Since one criterion of the suitability of an enzyme preparation is the completeness of the hydrolysis of cocarboxylase, this reaction was given careful study.

In order to simulate as closely as possible the actual conditions which would be encountered in the enzymatic hydrolysis of an unknown sample, 20 cc. of a pure solution containing 10.47 micrograms of cocarboxylase were placed in an extraction tube to which were added 30 cc. of 0.04 *N* sulfuric acid. The solution was heated in the usual manner for 1 hour and at the end of that time each stirrer was washed down with 5 cc. of distilled water and the tubes were cooled to room temperature. To each tube were then added 10 cc. of the enzyme solution. In the case of takadiastase and clarase, a 5 per cent solution was used, but as the two mylase preparations were less soluble, a lower concentration of these enzymes was employed, as shown in Table I. All the enzyme solutions were prepared in a sodium acetate–acetic acid buffer as previously described, so that the cocarboxylase solution, after the addition of the enzyme preparation, would have a pH of 4.5. The tubes were incubated for 2 hours at 45° C. with frequent stirring. At the conclusion of the incubation period, each stirrer was washed down with 3 cc. of distilled water, a 5-cc. aliquot was pipetted out of the solution, and a determination was made of its thiamin content using the procedure previously described. Control solutions containing only the enzyme preparations were run parallel to the test solutions, so that a correction could be made for any small amounts of the vitamin possibly present in the enzyme preparations.

From Table I it appears that either takadiastase, clarase, or the high-phosphatase mylase preparation can be employed for the hydrolysis of the cocarboxylase. Subsequent experiments have shown that, in most instances, a 2 per cent solution of clarase is as effective as the five per cent solution. As all the enzyme preparations are essentially diastatic, their activity toward cocarboxylase would appear to be due to the presence of an associated phosphatase. The starch-splitting property of the enzymes, however, is of value in obtaining a clear extract of the cereal products.

Table I. Hydrolysis of Cocarboxylase by Enzyme Preparations

Enzyme Solution Used	Yield of Thiamin in 5-Cc. Aliquot		
	Theoretical	Actual	Hydrolysis
	Microgram	*Microgram*	%
5% takadiastase	0.77	0.77	100
5% clarase	0.77	0.77	100
1% mylase (high phosphatase)	0.77	0.76	97
0.5% mylase (normal)	0.77	0.56	73

Influence of Amount of Alkali on Oxidation of Thiamin to Thiochrome. As various amounts of sodium hydroxide have been recommended for the oxidation of thiamin to thiochrome, a study of the effect of this variable was undertaken.

Solutions of thiamin chloride (1 cc. = 1 microgram) at pH values of 0.4, 2.2, and 4.7 were used. The solution at a pH of 2.2 was made up in 25 per cent potassium chloride to simulate the solution which would be obtained after the potassium chloride elution of the Decalso column. Solutions of sodium hydroxide were prepared, containing per cc. 0.001 gram of potassium ferricyanide, and 0.075, 0.15, 0.30, 0.45, and 0.60 gram, respectively, of sodium hydroxide.

To 1-cc. quantities of each of these sodium hydroxide–potassium ferricyanide solutions were added 0.5, 1.0, and 2.0 cc. of the thiamin chloride solution (representing 0.5, 1.0, and 2.0 micrograms of the vitamin) and the resulting solutions were made up to 5 cc. with the 25 per cent potassium chloride solution. To each 5-cc. portion, contained in a separatory funnel, were added 20 cc. of isobutyl alcohol and the oxidation was carried out in the manner previously described.

Table II shows that the amount of sodium hydroxide used plays an important role in the oxidation of thiamin to thiochrome. The results indicate that 1 cc. of a solution of sodium hydroxide containing 0.45 gram of the alkali and 0.001 gram of potassium ferricyanide was required to obtain maximum oxidation of thiamin chloride solutions containing from 0.5 to 2.0 micrograms of the vitamin, irrespective of the pH of the solution. The oxidation, as indicated by the galvanometer readings, is not further enhanced by using an amount of sodium hydroxide in excess of 0.45 gram per cc.

Table II. Effect of Amount of Sodium Hydroxide on Oxidation of Thiamin to Thiochrome

NaOH Added, Gram	pH of Thiamin Solution	Galvanometer Readings[a] Obtained from Indicated Amounts of Thiamin		
		0.5 microgram	1.0 microgram	2.0 micrograms
0.075	0.4	11.0	4.0	4.0
0.150	0.4	14.0	24.0	4.0
0.300	0.4	15.7	29.0	44.5
0.450	0.4	16.0	29.3	54.0
0.600	0.4	15.9	29.5	53.3
0.075	2.2	14.5	25.9	44.9
0.150	2.2	14.3	25.6	44.7
0.300	2.2	15.5	26.6	50.0
0.450	2.2	16.1	29.0	54.1
0.600	2.2	16.0	29.0	54.5
0.075	4.7	14.3	24.0	43.0
0.150	4.7	14.2	23.8	43.0
0.300	4.7	15.7	26.9	50.1
0.450	4.7	16.3	29.4	54.4
0.600	4.7	16.1	29.5	53.8

[a] Obtained with Pfaltz & Bauer fluorophotometer.

Influence of Amount of Potassium Ferricyanide. Having determined the amount of sodium hydroxide required in the oxidation of thiamin to thiochrome, the effect on the oxidation of varying the amount of potassium ferricyanide was next studied.

For this purpose, samples of the same thiamin chloride solutions were employed as for the study of the effect of alkali variation. Solutions of potassium ferricyanide were prepared containing per cc. 0.45 gram of sodium hydroxide and 0.0005, 0.001, 0.002, and 0.003 gram, respectively, of potassium ferricyanide. One cubic centimeter of each solution was then added to 0.5, 1.0, and 2.0 cc. of the thiamin chloride solution, representing 0.5, 1.0, and 2.0 micrograms of the vitamin, and the resulting solution in each case was made up to 5 cc. with the 25 per cent potassium chloride solution. After adding 20 cc. of isobutyl alcohol, the oxidation was carried out in exactly the same manner as in the sodium hydroxide study, with results shown in Table III.

Table III. Effect of Amount of Potassium Ferricyanide on Oxidation of Thiamin to Thiochrome

$K_3Fe(CN)_6$ Added	pH of Thiamin Solution	Galvanometer Readings[a] Obtained from Indicated Amounts of Thiamin		
		0.5 microgram	1.0 microgram	2.0 micrograms
Gram				
0.0005	0.4	16.1	28.9	53.9
0.0010	0.4	16.0	29.3	54.0
0.0020	0.4	16.2	29.2	55.0
0.0030	0.4	16.0	29.0	54.0
0.0005	2.2	16.5	29.8	54.9
0.0010	2.2	16.1	29.0	54.1
0.0020	2.2	16.0	29.5	54.9
0.0030	2.2	16.0	29.0	54.0
0.0005	4.7	16.0	29.0	54.1
0.0010	4.7	16.3	29.4	54.4
0.0020	4.7	16.2	29.0	56.0
0.0030	4.7	16.0	29.0	56.0

[a] Obtained with Pfaltz & Bauer fluorophotometer.

When the amount of sodium hydroxide is optimal, varying the amount of potassium ferricyanide has little effect on the oxidation of thiamin to thiochrome except in the case of the thiamin chloride solution at a pH of 4.7 containing 2 micrograms of the vitamin. In general, it may be concluded that 0.45 gram of sodium hydroxide and 0.002 gram of potassium ferricyanide will give a maximum oxidation of thiamin to thiochrome under any of the experimental conditions herein studied. From Tables II and III, the conclusion may also be drawn that if these amounts of sodium hydroxide and potassium ferricyanide are employed, the presence of potassium chloride is without influence on the reaction. This is of importance in that it shows that the standard solution of thiamin chloride, used for comparison with the unknown sample, does not need to be prepared in a 25 per cent solution of potassium chloride.

Influence of Amount of Isobutyl Alcohol. Having established the optimal amounts of soduim hydroxide and potassium ferricyanide to be employed in the oxidation of thiamin to thiochrome, a study was next undertaken to determine the effect of the amount of isobutyl alcohol used in the extraction of the thiochrome formed in the reaction.

For this investigation, a solution of thiamin chloride (1 cc. = 1 microgram) prepared in 25 per cent potassium chloride was employed. To 0.5, 1.0, and 2.0 cc. of this thiamin chloride solution, representing 0.5, 1.0, and 2.0 micrograms of the vitamin, was added 1 cc. of the sodium hydroxide–potassium ferricyanide solution containing 0.45 gram of the alkali and 0.002 gram of potassium ferricyanide. The resulting solution in each case was made up to 5 cc. with the 25 per cent potassium chloride solution.

To each solution, contained in a separatory funnel were added either 13, 15, or 20 cc. of isobutyl alcohol and the separatory funnel was shaken for 2 minutes on a mechanical shaking machine. After centrifuging and separating the aqueous layer, each solution was made up to contain 20 cc. of isobutyl alcohol. To each separatory funnel were then added 2 grams of anhydrous sodium sulfate, the separatory funnels were centrifuged, and a 15-cc. aliquot was pipetted into the cuvette of the fluorophotometer for a fluorescent reading.

Table IV. Effect of Amount of Isobutyl Alcohol

[On extraction of thiochrome formed in oxidation of thiamin by NaOH-$K_3Fe(CN)_6$]

Amount of Thiamin Chloride	Galvanometer Readings from Indicated Amounts of Isobutyl Alcohol		
	13 cc.	15 cc.	20 cc.
Micrograms			
0.5	16.0	15.9	16.1
1.0	28.4	29.0	29.0
2.0	54.0	54.9	54.9

Table IV shows that 15 cc. of isobutyl alcohol gave a maximum extraction of the thiochrome formed from amounts of thiamin chloride varying from 0.5 to 2.0 micrograms. In the authors' routine procedure, however, the use of 20 cc. of isobutyl alcohol has been found preferable, as this will allow a larger volume of liquid to be placed in the cuvette of the fluorophotometer for the fluorescence readings. The use of alcohols other than isobutyl alcohol for the extraction of the thiochrome is now being studied and will be reported in a subsequent paper.

Effect of Duration of Shaking on Extraction of Thiochrome Formed in Oxidation of Thiamin. For the study of this variable, the same thiamin chloride solution and the same experimental conditions were employed as in the foregoing. In each instance, 20 cc. of isobutyl alcohol were used in the extraction and the separatory funnels were mechanically shaken for intervals of 1, 1.5, 2, and 3 minutes. The results are given in Table V.

Table V. Effect of Duration of Shaking on Extraction of Thiochrome by Isobutyl Alcohol

Amount of Thiamin Chloride	Galvanometer Readings for Indicated Intervals of Shaking			
	1 min.	1.5 min.	2 min.	3 min.
Micrograms				
0.5	16.1	16.1	16.1	16.1
1.0	29.2	29.5	29.0	29.0
2.0	54.0	54.4	54.9	52.1

For amounts of thiamin chloride ranging from 0.5 to 1.0 microgram of thiamin chloride, the interval of shaking was without effect on the extraction of the thiochrome by 20 cc. of isobutyl alcohol. For solutions of thiamin chloride containing 2 micrograms, the shaking time should not exceed 2 minutes, as beyond that time a decrease in the galvanometer readings occurred. Since in most routine analyses the concentration of thiamin chloride in the mixture being oxidized does not exceed 1 microgram, a 1-minute shaking will prove sufficient.

Degree of Conversion of Thiamin to Thiochrome. Having established the optimal conditions for conducting the oxidation of thiamin to thiochrome, the degree of conversion of thiamin to thiochrome was studied.

For this study, a solution of pure thiochrome was prepared in 25 per cent potassium chloride. Precautions were taken to protect the solution from light destruction prior to its measurement. Amounts of this solution theoretically corresponding to 0.5, 1.0, and 2.0 micrograms of thiamin chloride were introduced into a separatory funnel, which was then shaken for 1 minute with 20 cc. isobutyl alcohol and 1 cc. of the sodium hydroxide–potassium

ferricyanide mixture containing 0.45 gram of the alkali and 0.002 gram of potassium ferricyanide. The resulting solution was centrifuged, treated with anhydrous sodium sulfate, and its fluorescence determined in the usual manner.

Table VI. Conversion of Thiamin to Thiochrome

Thiamin Chloride and Theoretical Thiochrome Equivalent	Galvanometer Reading	Conversion
		%
Thiamin chloride (0.5 microgram)	16.1	
Thiochrome (0.38 microgram)	24.3	67
Thiamin chloride (1.0 microgram)	29.3	
Thiochrome (0.77 microgram)	44.2	66
Thiamin chloride (2.0 micrograms)	54.4	
Thiochrome (1.54 micrograms)	81.0	67

The data of Table VI suggest that within the range of 0.5 to 2.0 micrograms of thiamin chloride, the degree of conversion of the thiamin to thiochrome is approximately 67 per cent. This is in confirmation of the results obtained by Ferrebee and Carden (*1*). However, the degree of solubility in isobutyl alcohol of the thiochrome formed in the oxidation of the thiamin is not known. It is possible that the low "conversion" values may in part be due to solubility relationships rather than to incompleteness of the oxidation itself. This point is under further investigation.

Comparison of Chemical Results with Bioassay Values. The reliability of the present method has been judged by comparing the results obtained on certain food materials with those obtained by biological assay on the same samples, using a rat-growth method. The results of such a comparison are shown in Table VII.

Table VII. Comparative Results Obtained from Chemical and Bioassay Procedures

Materials	Thiamin Content	
	Bioassay	Chemical
	Micrograms/gram	*Micrograms/gram*
Processed cereal product No. 1	3.15	3.27
Processed cereal product No. 2	1.12	1.26
Frozen peas	1.37	1.44

It is apparent that there is a close agreement between the chemical and biological assays and that the values obtained by chemical assay fall well within the range of the error inherent in the biological method.

Table VIII. Percentage Recovery of Thiamin Added to Various Samples

Material	Thiamin Content of Sample	Thiamin Added[a]	Amount of Added Thiamin Recovered	Recovery
	Micrograms/Gram	*Micrograms*	*Micrograms*	%
Processed cereal No. 5	3.15	4.00	3.61	90.00
Processed cereal No. 6	3.36	4.00	3.58	89.50
Processed cereal No. 7	1.56	8.00	7.96	99.50
Processed cereal No. 8	0.30	6.00	5.80	96.00
Skim milk powder	3.36	2.00	1.98	99.00
Wheat	3.90	2.00	2.00	100.00
Frozen spinach	1.26	2.00	1.92	96.00
Frozen peas	2.97	2.00	1.86	93.00
Frozen broccoli	0.84	2.00	1.86	93.00

[a] Thiamin added to initial extraction mixture.

Degree of Recovery of Thiamin Chloride Added to Food Products. In order to determine the degree to which the vitamin was preserved throughout the determination, a study was made of the percentage recovery of thiamin chloride added to various samples with the results given in Table VIII. The recovery of added thiamin is seen to be 90 per cent or better.

Table IX. Thiamin Content of Various Substances

Material	Thiamin Content or Range
	Micrograms/Gram
Barley	4.86–5.28
Fortified flour	1.56
Wheat germ	34.68
Wheat	1.05–6.87
Whey powder	1.69
Yeast	187.00
Skim milk powder	3.87
Cocoa	0.84
Green coffee	2.10
Fresh lima beans	2.97
Fresh broccoli	1.26
Fresh string beans	0.93
Fresh squash	1.47
Frozen peas	1.37–4.32

Thiamin Content of Various Food Products. The method has been successfully applied to various food materials, including cereals and fresh and frozen vegetables. Typical results obtained on various types of foodstuffs are given in Table IX.

Summary

A study of the thiochrome method for determining thiamin showed that, by the procedure developed, extraction and hydrolysis of the sample may be carried out in the same vessel, thereby eliminating any error due to transfer of the extract inherent in previous methods.

For the enzymatic hydrolysis of cocarboxylase, the enzyme clarase has been introduced.

From a study of the oxidation of thiamin to thiochrome, it was found that the most important factor was the amount of sodium hydroxide employed. One cubic centimeter of a solution of sodium hydroxide–potassium ferricyanide containing 0.45 gram of sodium hydroxide and 0.002 gram of potassium ferricyanide gave an optimal oxidation of thiamin to thiochrome for solutions of thiamin chloride containing from 0.5 to 2.0 micrograms of the vitamin. These amounts of sodium hydroxide and potassium ferricyanide gave an optimal oxidation of the thiamin to thiochrome, irrespective of the pH of the thiamin solution or of the presence or absence of potassuim chloride.

The optimal conditions for the extraction of the thiochrome formed in the oxidation of thiamin chloride solutions containing 0.5 to 2.0 micrograms of the vitamin have been found to be a 1-minute mechanical shaking in the presence of 20 cc. of isobutyl alcohol.

For amounts of thiamin chloride ranging from 0.5 to 2.0 micrograms, the conversion of thiamin to thiochrome is approximately 67 per cent under the conditions studied in this paper.

The method is in close agreement with biological assays, and has been applied to various types of natural products, including grains and fresh and frozen vegetables.

Acknowledgment

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Combined Determination of Riboflavin and Thiamin in Food Products

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NUMEROUS chemical and microbiological methods for the separate determination of thiamin and riboflavin have appeared in the literature. The procedure described below makes it possible to determine both vitamins on the same sample, and has enabled the authors to make complete analyses on as many as 8 to 12 samples in the course of a 7-hour day. The number is limited only by the extraction units in operation and available personnel. The method is an extension of the one proposed for thiamin (*2*), which includes a review of the literature on the determination of this vitamin.

Following the discovery by Kuhn and Wagner-Jauregg (*9*) that riboflavin concentrates give off a yellow-green fluorescence upon activation by light of certain wave lengths, numerous methods were proposed for determining the vitamin, based upon measurement of the fluorescence of riboflavin or of its degradation products, such as lumichrome and lumiflavin. In the earlier methods, the riboflavin was extracted with an organic solvent such as acetone or methyl alcohol and adsorbed on a fuller's earth preparation. The vitamin was then eluted with a mixture of methyl alcohol, pyridine, and water, and the fluorescence of the resulting solution determined visually or by means of a photocell as described by Cohen (*1*).

In 1935 Koschara (*7*) proposed a method based on measurement of the light absorption of the vitamin extract rather than on its fluorescence. Prior to measuring its light absorption, Koschara treated the extract with potassium permanganate to oxidize interfering pigments, and destroyed excess potassium permanganate with hydrogen peroxide.

Recently Hodson and Norris (*5*) proposed a method in which 0.25 *N* sulfuric acid was used to extract the riboflavin. Following the extraction, the solution was filtered, and the filtrate was brought to a pH of 7 with a phosphate buffer and treated with a mixture of sodium hyposulfite and stannous chloride. The authors stated that sodium hyposulfite reduces the riboflavin, while stannous chloride reduces the interfering pigments. The riboflavin was then reoxidized by bubbling air through the solution and its fluorescence measured by the use of a fluorophotometer.

Ferrebee (*3*) has recently introduced two fuller's earth preparations, Floridin and Supersorb, for the adsorption of riboflavin. The vitamin was eluted from these adsorbents with a solution of 20 per cent pyridine in 2 per cent acetic acid, and the eluate treated with potassium permanganate and hydrogen peroxide prior to measuring the fluorescence. Other methods (*4, 10–13*) based on the above procedures have been proposed.

Description of Method

The sensitivity of riboflavin to light makes it necessary to carry out the entire analysis under controlled illumination. The authors used Eastman safety lights equipped with series OA Wrattan light filters.

For the combined determination of thiamin and riboflavin, a 3- to 5-gram sample of the finely pulverized food material is prepared as described in the thiamin method (*2*). The weighed sample is placed in the specially designed extraction tube (*2*) and is extracted with 50 cc. of 0.04 *N* sulfuric acid on a boiling water bath for approximately 1 hour, with continuous stirring. At the conclusion of the extraction, each stirrer is carefully washed down with 5 cc. of distilled water delivered from a graduated pipet and the tube is cooled to room temperature with running water. Ten cubic centimeters of a five per cent solution of clarase (*2*) are added to each tube and the contents are thoroughly mixed with a glass stirring rod. The tube, containing the stirring rod, is then incubated at 45° C. for 2 hours. Following the incubation period, each stirring rod is carefully washed down with exactly 1 cc. of distilled water and the extraction tube is cooled to room temperature. Each tube is then centrifuged at high speed until a clear supernatant liquid is obtained.

The aliquot to be taken from the supernatant liquid will be dependent upon the amounts of thiamin and riboflavin present in the extract. A single aliquot may be used if it contains about 5 micrograms of thiamin and 1 microgram of riboflavin. Otherwise, separate aliquots should be taken for the determination of each vitamin. The adsorption apparatus used for the combined determination of riboflavin and thiamin consists of two glass adsorption columns (*2*), connected by a No. $6^1/_2$ two-hole rubber stopper so that the filtrate from the upper column falls directly onto the lower column. The stopper is provided with a small one-way glass stopcock to regulate the flow of liquid through the upper column. The combined columns are attached to a modified vacuum receiver (Scientific Glass Apparatus Co., Bloomfield, N. J.) connected to the laboratory suction outlet. Figure 1 represents a single unit and Figure 2 a multiple assembly.

The upper column is filled with activated 60- to 80-mesh Decalso, and the lower column with 60- to 80-mesh Supersorb prepared as described by Ferrebee (*3*). In his original method the adsorption of riboflavin was carried out with a column 15 cm. long and 1 cm. in diameter filled with 30- to 60-mesh Supersorb. However, it was found that if 60- to 80-mesh Supersorb were used, a column of design similar to that used for the adsorption of thiamin could be employed.

The solution containing the riboflavin and thiamin is allowed to pass through the two columns at the rate of 1 cc. per minute, the flow being regulated by adjusting the stopcock between the two columns as well as that on the vacuum receiver. (The stopcock on the vacuum receiver is lubricated with glycerol or orthophosphoric acid to avoid fluorescent impurities present in other lubricants.) Thiamin is adsorbed on the Decalso, but the riboflavin passes through it and is adsorbed on the lower column of Supersorb. Both columns are washed down thoroughly with hot distilled water and the washings are discarded. The upper column containing the Decalso is then detached and transferred to a 125-cc. glass suction flask where it is ready for elution of the thiamin with 25 per cent potassium chloride as previously outlined (*2*).

FIGURE 1

The lower column containing the Supersorb is detached from the vacuum receiver, which is washed with hot water and dried by pulling air through it. The column is then attached to the receiver again and the riboflavin eluted with 25 cc. of a solution of 20 per cent pyridine in 2 per cent acetic acid added from an automatic pipet, the flow through the column being adjusted to 1 cc. per minute. (The pyridine solution should have a low fluorescence and be stored in a dark bottle.) Additional small portions of the eluant are then passed through the column until the volume of the filtrate is approximately 50 cc. as indicated by the graduated readings on the vacuum receiver. In analyzing most food materials, the filtrate is then made up to exactly 50 cc. with the pyridine–acetic acid mixture. However, for concentrates of riboflavin it may be necessary to make up the filtrate to a larger volume, so that a 15-cc. aliquot will contain from 0.5 to 1.0 microgram of the vitamin.

A 15-cc. aliquot of the riboflavin filtrate is pipetted into a 50-cc. brown bottle or flask, 1 cc. of a 4 per cent solution of

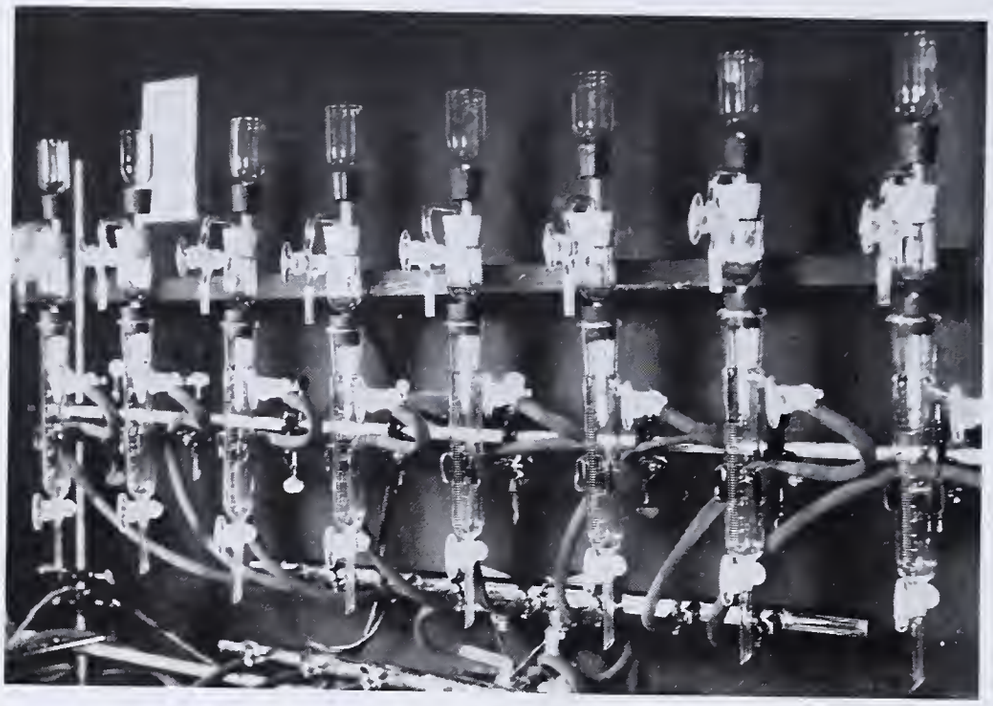

FIGURE 2. MULTIPLE ASSEMBLY OF ADSORPTION APPARATUS

TABLE I. DESTRUCTION OF RIBOFLAVIN BY LIGHT AT VARIOUS pH VALUES

Nature of Illumination	pH of Riboflavin Solution	Loss of Riboflavin Following Different Intervals[a]			
		1 day	2 days	5 days	7 days
		%	%	%	%
Diffused daylight	2.2	40	96	98	..
	4.0	84	96	96	..
	6.0	82	94	98	..
	8.0	72	96	96	..
Artificial light	2.2	12	16	16	29
	4.0	15	37	60	78
	6.0	16	38	58	79
	8.0	23	37	55	65
Darkness	2.2	..	..	..	0
	4.0	..	..	..	0
	6.0	..	..	..	0
	8.0	..	..	..	0

[a] Initial content of riboflavin in each solution was approximately 50 micrograms.

potassium permanganate is added and, after an interval of a minute, 3 cc. of a 3 per cent solution of hydrogen peroxide. The latter can be conveniently prepared by diluting a 30 per cent solution of hydrogen peroxide (Superoxol) a few minutes before the determination is to be run. The potassium permanganate solution should be prepared fresh daily. The reaction mixture is shaken vigorously and then allowed to stand until all effervescence has ceased, as the presence of bubbles will introduce an error in the fluorescence readings. Fifteen cubic centimeters of the solution are pipetted into a cuvette of the fluorophotometer, and fluorescence readings are made after adjusting the instrument (*2*). A Corning glass filter No. 511 is used for transmitting the incident light and No. 351 for the fluorescent light. Both filters are of 2-mm. thickness.

Although in the range 0.2 to 2.0 micrograms a linear relationship exists between the amount of riboflavin and galvanometer readings, it has been found advisable to make a daily determination on a standard solution of riboflavin in pyridine–acetic acid instead of depending entirely upon a calibration curve. The standard solution is made up to contain 0.0856 microgram of riboflavin per cc. and should be kept in a dark-colored bottle away from light. No destruction of the vitamin occurs under these conditions.

For determining the fluorescence of the standard solution, a 15-cc. aliquot is treated with 1 cc. of the 4 per cent solution of potassium permanganate and 3 cc. of 3 per cent hydrogen peroxide. Fifteen cubic centimeters of this solution, representing 1 microgram of the vitamin, are then pipetted into the cuvette of the fluorophotometer and its fluorescence is determined. The standard solution must be treated in the same manner as the sample, since the fluorescence of riboflavin solutions is dependent upon the solvent employed as well as upon the hydrogen-ion concentration. A blank correction for the standard solution is obtained by determining the fluorescence of 15 cc. of a solution taken from a mixture of 1 cc. of 4 per cent potassium permanganate, 3 cc. of 3 per cent hydrogen peroxide, and 15 cc. of the pyridine–acetic acid reagent.

A "complete blank" (*2*), consisting of all the reagents used throughout the method, is also run through the entire procedure and serves as a blank for the thiamin determination as well as for the riboflavin. In the case of riboflavin the value for the blank is subtracted from fluorescence readings of the sample.

Critical Study of Steps in Procedure

EFFECT OF LIGHT ON RIBOFLAVIN SOLUTIONS AT VARIOUS pH VALUES. As various investigators had reported that riboflavin is sensitive to light, the authors studied the extent of destruction caused by light and whether it is influenced by the pH of the solution.

Solutions of pure riboflavin were prepared in phosphate–citric acid buffers to give pH values ranging from 2 to 8. The solutions were placed in white glass bottles having tight screw caps, and a layer of toluene was added to prevent mold growth. One set of solutions was exposed to the diffused light of the laboratory, another set to artificial light, and a control set was kept in a dark room. The amount of riboflavin present in each solution was determined fluorometrically at the beginning of the experiment and at frequent intervals thereafter, with the results given in Table I. As Karrer and Fritzsche (*6*) and Kuhn and Moruzzi (*8*) have shown that the hydrogen-ion concentration affects the fluorescence of riboflavin solutions, all the determinations were conducted at the same pH value. This was accomplished by pipetting a 1-cc. aliquot from each of the test solutions into a phosphate–citric acid buffer of pH 6.0. A suitable aliquot (15 cc.) of this buffered solution was used for fluorometric analysis.

From Table I, it will be seen that 1 day's exposure to the diffused light of the laboratory caused an extensive destruction of riboflavin, particularly in the samples at pH values above 2.2. After 5 days' exposure, practically none of the original riboflavin remained in any of the solutions. In the solutions exposed to artificial illumination, the destruc-

TABLE II. ADSORPTION OF THIAMIN AND RIBOFLAVIN BY DECALSO

Vitamin Content of Solution			
Before Adsorption		After Passing through Decalso Column	
Thiamin	Riboflavin	Thiamin	Riboflavin
Micrograms	*Micrograms*	*Microgram*	*Micrograms*
5.00	3.00	0	3.06
5.00	5.00	0	4.95
5.00	10.00	0	9.92

TABLE III. ELUTION OF RIBOFLAVIN FROM ADSORBENTS WITH PYRIDINE–ACETIC ACID MIXTURE

Amount of Riboflavin, Micrograms	Galvanometer Readings on Riboflavin Solution[a]		
	Control	After elution from Floridin	After elution from Supersorb
5	28.0	25.7	28.1
10	47.2	42.0	47.6
15	66.0	58.8	66.3

[a] Obtained on Pfaltz & Bauer fluorophotometer.

Table IV. Corning Glass Filter Combinations for Fluorometric Determination of Riboflavin

Incident Light Filter[a]	Fluorescent Light Filter[a]	Galvanometer Readings: Riboflavin solution	Galvanometer Readings: Blank
511 + 038	368	72	18
511 + 038	038 + 368	62	12
511 + 038	351	32	2
511 + 038	038 + 351	29	2
511 + 038	440 + 401	17	6
511	440 + 401	37	7
511	351	73	5
511	351 + 428	40	3
584	440 + 401	6	2
584	351	15	2
584	368	26	2

[a] Filter numbers are those used by Corning Glass Works.

Table V. Comparative Results Obtained by Chemical and Bioassay Procedures

Material	Riboflavin Content: Bioassay	Riboflavin Content: Chemical
	Micrograms/gram	*Micrograms/gram*
Processed cereal No. 1	2.50	2.29
Frozen peas	1.55	1.45

tion proceeded at a slower rate. At higher pH values the destruction is particularly rapid. No loss occurred in any of the control solutions.

Adsorption Studies with Riboflavin. An essential feature of this procedure is the use of a single extract of the food material for the combined determination of riboflavin and thiamin. This involves the assumption that none of the riboflavin is adsorbed on the Decalso column.

To test this assumption, solutions containing 5 micrograms of thiamin and varying amounts of riboflavin were prepared at a pH value (4.5) corresponding to that of the food extract following enzymatic hydrolysis. Each solution was then passed through the activated Decalso column in the usual manner and the amount of riboflavin adsorption determined.

The results given in Table II show that no appreciable adsorption of riboflavin from solutions containing 3 to 10 micrograms occurred on the Decalso column.

A study was also made of the adsorption of riboflavin on the two fuller's earth preparations (Floridin and Supersorb).

Both adsorbents were reduced to 60- to 80-mesh and activated according to Ferrebee's directions (*3*). Solutions containing various amounts of riboflavin at pH 4.5 were passed through columns of each adsorbent at the rate of 1 cc. per minute. Each column was then washed thoroughly with hot distilled water and finally eluted with 25 cc. of the pyridine–acetic acid mixture. Fluorescence readings were made on the eluates and on control solutions of riboflavin in pyridine–acetic acid containing the same amounts of the vitamin as originally passed through the columns.

The results obtained are given in Table III. Although the recovery of riboflavin from the Supersorb was excellent, the recovery from Floridin was incomplete. As it was subsequently established that adsorption of riboflavin was quantitative on both adsorbents, the low recovery from Floridin is apparently due to failure to obtain complete elution of the riboflavin from this column with 25 cc. of the pyridine–acetic acid mixture. Therefore, the Supersorb was selected for use in the method.

Selection of Light Filters for Determination of Riboflavin. Various Corning glass filters were tested in the fluorophotometer to determine their adaptability for transmitting the required incident light and fluorescent light. In making the tests, 15 cc. of an aqueous solution of riboflavin, containing 1 microgram of the vitamin, were placed in the cuvette of the fluorophotometer and its fluorescence was determined using various filter combinations. Readings were also made on a blank consisting of 15 cc. of distilled water. The results are shown in Table IV.

The combination of filter 511 for transmission of the incident light with filter 351 for the fluorescent light gave the highest reading with the lowest blank, and consequently these were selected for use. For activating the riboflavin to fluorescence, filter 511, transmitting light of approximately 445 mμ, is superior to 584 which transmits ultraviolet light. Although filter 368 gives a high fluorescence reading, there is also a correspondingly large blank, indicating transmission of wave lengths other than those derived from the fluorescence of the riboflavin.

Comparison of Results by Bioassay and Chemical Procedures

In order to test the validity of the method for determining riboflavin, bioassays were run on certain samples using a rat-growth method, with the results given in Table V. In conducting the bioassays, the following basal diet was employed:

Labco vitamin-free casein, %	20
Cornstarch, %	67
Osborne-Mendel salt mixture, %	4
Hydrogenated fat (Primex), %	4
Cod liver oil, %	2
Labco rice polish concentrate No. 2, %	2
Thiamin chloride, 20 micrograms per rat per day	
Pyridoxin, 10 micrograms per rat per day	

From Table V, it is apparent that the chemical values are in good agreement with those obtained by bioassay, and that the difference in results is well within the range of error inherent in the biological method.

Table VI. Percentage Recovery of Added Riboflavin

Sample	Riboflavin Content of Sample	Riboflavin Added	Recovery of Added Riboflavin	Recovery
	Micrograms/gram	*Micrograms/gram*	*Micrograms*	%
Wheat germ	5.56	2.50	2.49	99
Wheat	1.74	4.00	3.65	91
Yellow corn	1.16	2.00	1.93	99

Recovery of Riboflavin Added to Foodstuffs

In order to test the reliability of the method, various amounts of pure riboflavin were added to food extracts and percentage recovery of the vitamin was determined, with the results shown in Table VI. The recovery of added riboflavin was over 90 per cent in each case, which indicates that the procedure does not lead to appreciable loss of the vitamin.

Table VII. Riboflavin Content of Foodstuffs

Material	Riboflavin Content or Range
	Micrograms/gram
Wheat	0.89–2.03
Yellow corn	0.83–2.02
White corn	1.27–2.29
Rice polishings	2.52
Rice bran	2.41
Wheat germ	3.78–5.56
Wheat bran	3.17
Skim milk powder	19.00–29.00
Whey powder	59.00
Frozen peas	1.90
Frozen broccoli	2.00
Fresh spinach	1.17–1.47
Fresh lima beans	1.35–1.56
Fresh broccoli	1.76
Fresh string beans	1.61

Riboflavin Content of Foodstuffs

The method has been applied to various food materials with the results shown in Table VII. These values are in agreement with those reported by Hodson and Norris (*5*) for similar materials.

Summary

A rapid and accurate method has been developed for determination of thiamin and riboflavin on the same sample.

The method is in close agreement with biological assays and has been applied to grains, milk products, and fresh and frozen vegetables.

A study of the destruction of riboflavin by light in aqueous solutions at pH values ranging from 2 to 8 showed that rapid destruction of the vitamin occurred, irrespective of the pH, when the solutions were exposed to the diffused light of the laboratory. The destruction from exposure to artificial illumination was slower and appeared to be dependent upon the pH.

Ferrebee's procedure for the adsorption of riboflavin on Supersorb has been modified to use a smaller extraction column.

A study has been made of Corning glass filters suitable for the fluorometric determination of riboflavin. Filter 511 has been selected for transmitting the incident light and No. 351 for transmitting the fluorescent light.

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Ethylene Glycol Determination in and Removal from Commercial Alkyl Ethers of Diethylene Glycol

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IN THE course of work in the Research Laboratory of Organic Chemistry, samples of commercial ethyl and methyl ethers of diethylene glycol have been found to contain 28 and 8.5 per cent ethylene glycol, respectively. No previous report of the presence of this impurity has been located.

Two independent procedures for the determination of the amount of this ethylene glycol, the ditrityl ether method and the lead tetraacetate oxidation method (*3*), have now been developed and yield closely checking results. In the first, the glycol is converted into its ditrityl ether which can be separated from the other monotrityl ethers and by-products because of its relative insolubility and weighed; this determination is rapid and yields excellent proximate results. In the second, the glycol is oxidized with excess lead tetraacetate in acetic acid, the decrease in the oxidizing power of the solution serving as a direct measure of the glycol content for the alkyl ethers of diethylene glycol are substantially unaffected; this method requires a longer time and a more complex procedure, but gives more accurate results. The assumption that ethylene glycol is the only 1,2-glycol and the only easily oxidizable impurity present is supported by the close agreement between the two methods.

Removal of the ethylene glycol from commercial alkyl ethers of diethylene glycol by ordinary fractionation, the method of purification reported previously by Veraguth and Diehl (*6*) and Whitmore and Lieber (*7*), is very inefficient (almost ineffective in the case of the ethyl ether), owing to the proximity of the boiling points of ethylene glycol (197° C.), diethylene glycol monomethyl ether (191° C.), and diethylene glycol monoethyl ether (198° C.). It can be removed readily, however, by a solvent partition method using benzene and water.

Ditrityl Ether Method

Heat for 15 minutes on the steam bath in a test tube 0.25 ml. of diethylene glycol monomethyl or monoethyl ether with 1 to 2 ml. of pyridine and trityl chloride in excess over that amount calculated to convert all the ethylene glycol to its ditrityl ether and the diethylene glycol alkyl ether to its trityl ether. Leach with water, ice until the oil stiffens to a partially crystalline mass, and wash by decantation with several portions of fresh water. Extract the gum with 20 ml. of 95 per cent alcohol. The alcohol-insoluble (*5*) precipitate is the ditrityl ether of ethylene glycol and should melt around 170–180°; if it melts much lower, reextract. From the weight of this residue the per cent of glycol in the original can be calculated. In the present work the determinations shown in Table I were made.

Identity of the impurity as ethylene glycol was attested by the following facts: The alcohol-insoluble precipitate obtained above melted at 187° after two recrystallizations from acetone and did not lower the melting point of an authentic sample of ethylene glycol ditrityl ether (m. p. 187–187.5°).

TABLE I. ETHYLENE GLYCOL CONTENT OF COMMERCIAL ALKYL ETHERS OF DIETHYLENE GLYCOL

Monomethyl Ether	Ethylene Glycol: By ditrityl ether method	Ethylene Glycol: By lead tetraacetate method
	%	%
Commercial material, 1937	9.2, 8.6, 8.4	...
Commercial material, 1940	9.2, 8.4, 7.9	8.7, 8.6
Artificial mixture, 10.4% glycol	10.1, 10.1	...
Artificial mixture, 9.8% glycol	10.1, 11.1	...
Artificial mixture, 1.95% glycol	2.0, 1.7[a], 1.7, 1.9[b]	...
Highly purified (see below)[c]	Trace	...
Highly purified, refractionated	...	0.41–0.45[c] 0.42–0.45
Monoethyl Ether		
Commercial material, 1937, discolored	24, 27	...
Commercial material, 1937, colorless	28	28.0, 28.0
Commercial material, 1940	26, 26, 28	28.2, 28.2
1937 material dehydrated and distilled	28, 29	...
Artificial mixture, 27.2% glycol	25, 24, 24	...
1937 material partially purified	4.5, 6.3	...
1937 material highly purified	Less than 0.5	0.7–0.8, 0.7–0.8[d]
Monobutyl Ether		
Commercial material	Not more than a trace	...

[a] Using enough trityl chloride for 50% glycol.
[b] Using enough trityl chloride for 10% glycol.
[c] See paragraph, "Removal of Ethylene Glycol".
[d] Curves of % glycol plotted against time did not flatten out but drifted slowly upward. However, between limits given there was a definitely sharp change of slope.

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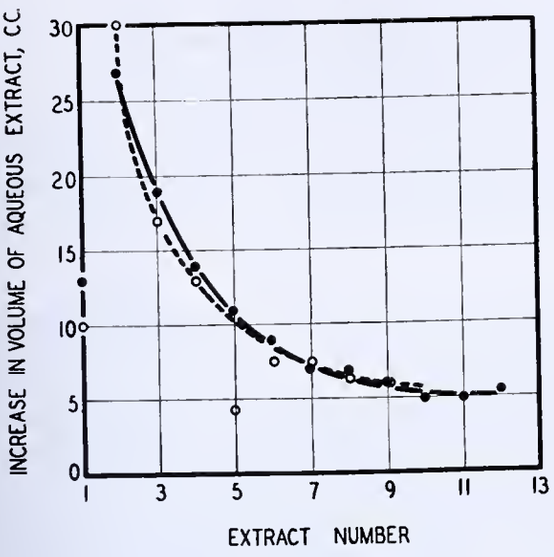

FIGURE I. AQUEOUS EXTRACTIONS OF A BENZENE SOLUTION OF COMMERCIAL DIETHYLENE GLYCOL MONOETHYL ETHER

●——● Run I
○- - -○ Run II, using two fifths of the quantities of Run I, increase in volume of extract multiplied by 2.5

TABLE II. FRACTIONATION OF COMMERCIAL ALKYL ETHERS OF DIETHYLENE GLYCOL

Monomethyl Ether Fraction	B. P.[a] °C.	Weight Grams	n_D^{25}	Glycol Content[b] %
Original	.....	..	1.4253	8.5
2	188–189.5	1.9	1.4255	..
3	189.5–191	1.8	1.4254	12.9
4	191–192	3.4	1.4262	13.1
5	192–193	12.6	1.4254	10.8
6	193	12.2	1.4247	2.5
7	193–193+	2.5	1.4246[c]	0
Monoethyl Ether				
Original	.....	..	1.4271	26
2	186–191	7.6	1.4285	35
3	191–194[d]	17.2	1.4281	28
4	194–196	2.8	1.4269	10
5	196–197.5	3.3	1.4256	1.8
6	197.5	2.1	1.4254[c]	0

[a] At 768 mm.
[b] By dirityl ether method.
[c] Same as values determined on highly purified material.
[d] One half at 191–191.5°.

Where insufficient trityl chloride was used, ethylene glycol monotrityl ether could also be obtained by adding petroleum ether to the residual oil (m. p. after purification 104.5–105°). It did not lower the melting point of an authentic sample (m. p. 105–105.5°), and analyzed correctly as follows: calculated for $C_{21}H_{20}O_2$: C, 82.9; H, 6.62. Found: C, 83.1; H, 6.80.

Lead Tetraacetate Oxidation Method

This was carried out essentially as described by Hockett (*3*), but simplified as follows, since only the end point of the reaction was important, not the rate. The reactions were allowed to proceed at room temperature, 28° to 33° C. At this higher temperature they were complete in 25 to 40 hours (instead of 50 to 100 hours, the time required at 20°), the reaction being considered complete when the amount of lead tetraacetate consumed either had ceased to increase or drifted upward only slowly. It was found advisable to run blanks simultaneously, for the strength of the lead tetraacetate solution decreased appreciably, probably because of incomplete removal of aldehydes from the acetic acid. The results are shown in Table I.

Fractionation of Commercial Materials

Commercial diethylene glycol monomethyl and monoethyl ethers (Carbide and Carbon Chemicals Corporation) were dried over anhydrous sodium sulfate and fractionated through a column with a glass spiral of the Podbielniak type of eight theoretical plates. Refractive indices and ethylene glycol content of the various fractions were determined. The results in Table II would suggest that while refractionation of fraction 6 diethylene glycol monomethyl ether would yield glycol-free material in fair amounts, it would be almost impossible to obtain large quantities of glycol-free diethylene glycol monoethyl ether by fractionation.

Removal of Ethylene Glycol

A solution of 250 grams of diethylene glycol monomethyl or monoethyl ether in 750 ml. of benzene was extracted with 5-ml. portions of water, allowing 10 minutes for separation. The volumes of the aqueous extracts were measured, the increase in volume was calculated, and the extraction was continued until this increase had become constant. Figure 1 illustrates the type of results obtained with the ethyl ether. Less satisfactory results were produced by the methyl ether; no layer was obtained until 25 ml. of water had been added, the increase in volume tended to be erratic, and the constant increase was about 12 to 14 ml. The residual benzene layers were dried over anhydrous sodium sulfate, the benzene was distilled off, and the residues were fractionated through the eight-plate column.

Diethylene glycol monoethyl ether: b. p. 196° C. (762 mm.), d_4^{25} 0.9855, n_D^{25} 1.4254, n_D^{20} 1.4273. The material was very hygroscopic; after 2 minutes' exposure of a drop to the air on a humid day the index at 25° fell to 1.4226. Analysis: Calculated for $C_6H_{14}O_3$: C, 53.7; H, 10.5. Found: C, 54.0; H, 10.8.

Diethylene glycol monomethyl ether: b. p. 190° C. (757 mm.), d_4^{25} 1.0161, n_D^{25} 1.4246, n_D^{20} 1.4263; refractionated, b. p. 191° (762 mm.), n_D^{25} 1.4246. This material was also hygroscopic; after 2 minutes' exposure of a drop to air on a humid day the index at 25° fell to 1.4214, after 4 minutes' exposure to 1.4189. Analysis: Calculated for $C_5H_{12}O_3$: C, 50.0; H, 10.1. Found: C, 49.8; H, 10.3.

Physical constants of purified or commercial samples of these compounds have been reported by Veraguth and Diehl (*6*), Whitmore and Lieber (*7*), Gardner and Brewer (*2*), Hofmann and Reid (*4*), and Davidson (*1*).

Approximately 30 per cent recoveries of the pure alkyl ethers of diethylene glycol may be obtained by this method.

Summary

Samples of commercial diethylene glycol monoethyl and monomethyl ethers have been shown to contain 28 and 8.5 per cent of ethylene glycol, respectively, by either of two independent methods, dirityl ether formation or lead tetraacetate oxidation.

A solvent partition method was devised for removing this glycol, since fractionation is relatively ineffective.

Acknowledgment

The author wishes to thank E. H. Huntress of the Massachusetts Institute of Technology for suggesting the solvent partition method of removing ethylene glycol and R. C. Hockett of Massachusetts Institute of Technology for mentioning the possibility of applying lead tetraacetate oxidation to the determination of ethylene glycol.

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PRESENTED before the Division of Organic Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich. Contribution 242 from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology.

Sampling and Analysis of Phosphorus

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AN IMPROVED apparatus and a procedure for sampling and analyzing phosphorus have been developed for routine control of the phosphorus condensing plant of the TVA Fertilizer Works located at Wilson Dam, Ala. (*2*). The grades of phosphorus which may be sampled and analyzed range from purified white phosphorus to sludges that contain phosphorus, furnace dust, gelatinous silica, crystals of potassium fluosilicate, and water. In the plant, all grades of phosphorus are maintained at temperatures above the melting point of phosphorus, so that they can be transferred from one unit to another by pumping. The phosphorus is stored under a seal of water.

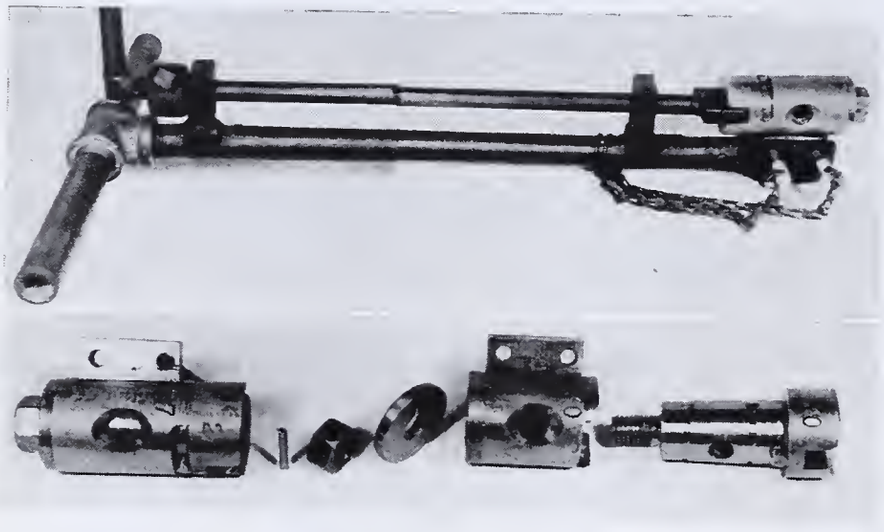

FIGURE 1. SAMPLER FOR LIQUID PHOSPHORUS
Above, attached to handle
Below, assembled and disassembled

The problem of determining the quality of phosphorus in process or in storage involves removal and preservation of representative samples from large-scale equipment, transfer of the samples to the analytical apparatus without either loss or oxidation, extraction of the phosphorus with an appropriate solvent, and determination of the phosphorus in the extract.

Brown, Morgan, and Rushton (*1*) reported the initial study made in this laboratory on the procedures for sampling and analyzing phosphorus. After these procedures had been used in plant control, certain improvements were made from time to time and are embodied in the apparatus and procedure described in this paper.

In the initial procedure, samples of phosphorus withdrawn from a storage tank by means of the usual types of thief or bottle samplers contained some of the seal water as well as the water dispersed in the phosphorus. To obtain representative samples with these samplers, a large sample was taken, placed under a seal of water, and later subdivided to obtain the sample used for analysis. Subdivision of the sample by the granulating technique described washed away a portion of the salts, dispersed the silica and fine particles of phosphorus, and modified the amount of dispersed water and the resultant granules, so that the sample taken for analysis was not representative of the material initially sampled. The error is slight when this technique is applied to pure phosphorus but it increases rapidly as the quality of the phosphorus becomes poorer.

Sampler

A sampler has been devised to overcome the major difficulties encountered in obtaining representative samples of phosphorus for analysis.

As shown in Figure 1, the sampler is essentially a brass stopcock wherein the sample is obtained by trapping a slug of phosphorus in the slightly tapered bore of the plug part of the stopcock. Detachable handles of different lengths are provided, so that tanks of various depths can be sampled conveniently. A dismantled sampler and a sampler attached to a handle are shown in Figure 1. The samplers are numbered to aid in keeping record of samples. A mixture of equal parts by volume of a heavy grease and white lead-in-oil paste is used for lubrication.

Sampling Procedure

The closed sampler, attached to a handle of proper length, is lowered into a tank of phosphorus to the level from which a sample is desired; the sampler is opened by turning the handle through a quarter turn and is swung a few times through a short arc to clear the bore of air or adhering sludge from a higher level. The sampler is then closed and withdrawn from the tank and its exterior is washed with a stream of water or steam. The handle is detached from the sampler and the operation is repeated with another sampler at another level in the tank.

The bore of the sampler holds approximately 3 grams of phosphorus. A few unit samples will represent accurately the quality of a tank of high-grade phosphorus, but many

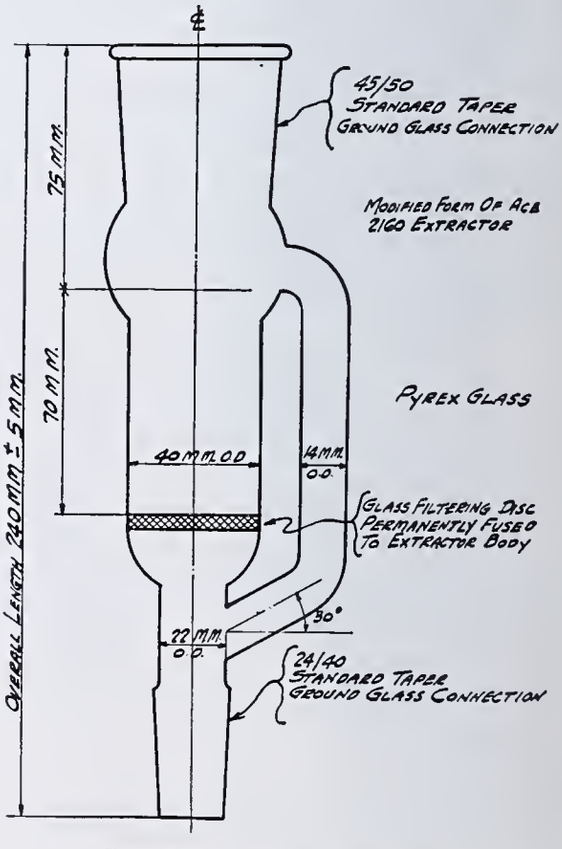

FIGURE 2. EXTRACTION TUBE

samples are necessary in determining the grade of phosphorus sludge. The extraction apparatus described below permits combination of a maximum of ten 3-gram samples for one analysis. One such analysis has been found to represent accurately a body of the higher grades of phosphorus. The unit samples of the composite sample should be taken so that each unit sample represents an equal volume of the phosphorus in the container.

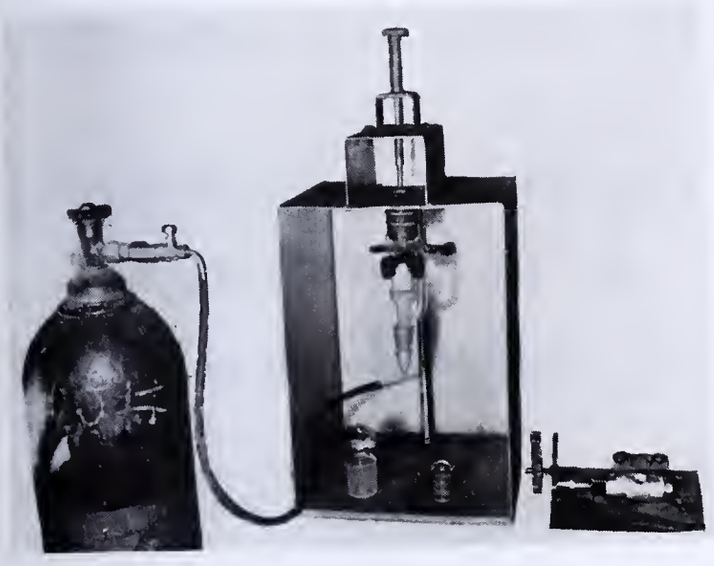

FIGURE 3. SAMPLER AND SAMPLE TRANSFER APPARATUS WITH EXTRACTION TUBE IN PLACE

Since the entire contents of the samplers are used for analysis, duplicate samples, when required, should be taken separately. The duplicate sample can be left in the sampler indefinitely without troublesome corrosion of the sampler. If the moisture content of the sample can be ignored, the unit samples can be transferred to a vessel containing water and later analyzed for total phosphorus and benzene-insoluble material.

Extractor

The extraction apparatus described by Brown, Morgan, and Rushton has been modified by replacing the crucible and flask with an extractor tube (Figure 2) and boiling flask. A sintered-glass disk of medium porosity supports the sample and retains the benzene-insoluble material. A disk with smaller pores will retain water from the sludges so tenaciously that the flow of benzene through the disk is impeded and the benzene overflows through the side arm, carrying with it insoluble material. The extractor tube is provided with a stopper for the large opening, and a cap and an adapter for the small opening. The apparatus, shown in Figure 3, is used to transfer the sample from the sampler to the extractor.

Transfer of Sample to Extractor

The extractor with stopper and cap is dried and weighed. The extractor is clamped in position, as shown, and then connected to a supply of nitrogen. The dry sampler is fastened in the holder with pins through the wing and placed in position over the extractor.

A stream of nitrogen is passed through the extractor at the rate of about 1 liter per minute for 2 to 3 minutes to clear the system of oxygen, and the lever is turned to open the sampler. If the slug does not drop into the extractor, the plunger is pushed through the sampler to remove it. High-grade phosphorus can be transferred completely from the sampler to the extractor, but films that adhere to the walls of the sampler complicate the transfer of low-grade sludges. The material lost in transfer is chiefly water. Although the weight of water lost in the sampler with each determination has not been measured, it is probably relatively constant for a given grade of sludge. Drainage from sludges is prevented from coming in contact with the ground surface of the top joint of the extractor by insertion of a conical metal shield.

After introduction of a sufficient number of slugs of phosphorus, the extractor is moved downward without stopping the flow of nitrogen, the upper stopper inserted, the adapter removed from the lower end, and the lower cap attached. The weight of the extractor and sample is determined by means of a balance accurate to 0.5 mg.

Extraction of Sample

After weighing the extractor and sample, the cap is removed, the flask containing benzene is attached, the stopper is removed, and the extractor is connected to the water trap. The complete assembly of the extraction apparatus is shown in Figure 4. The volume of the flask should be adequate to contain the benzene required at room temperature to dissolve at least one and one-half times the quantity of phosphorus in the sample, so that the benzene solution will not be more than two-thirds saturated. The solution should not contain over 2.4 grams of phosphorus per 100 ml. For routine work, a minimum amount of benzene for extraction of one 3-gram sample is 200 ml. in a 500-ml. flask. After completion of the extraction, the benzene solution of phosphorus is transferred to a volumetric flask of appropriate size (250 ml. for a 3-gram sample) and made to volume.

If, for any reason, the above ratio of phosphorus to benzene has been exceeded, so that the benzene solution is more than two-thirds saturated, there is danger of spontaneous ignition of the phosphorus solution when it is transferred to a volumetric flask. In this case the benzene solution may be left in the boiling flask, cooled, and diluted to mark. It is convenient to use the lower edge of the ground surface as a calibration mark on the boiling flask. To facilitate mixing, the neck of the boiling flask should be extended by means of a glass-stoppered adapter tube, thus simulating the usual volumetric flask.

The volume of water in the trap is read. The extractor is heated at 105° C. for an hour to remove the benzene; the extractor is then stoppered and weighed.

Typical results are as follows:

Weight of extractor + sample, grams	254.555
Weight of extractor, grams	251.750
Weight of sample, grams	2.805
Volume of water collected in trap, ml.	0.2
Weight of extractor + benzene-insoluble residue, grams	251.793
Weight of benzene-insoluble residue, gram	0.043
Benzene-insoluble, % of dry sample	1.6

Determination of Phosphorus in Benzene Solution

The determination of phosphorus in the benzene solution involves three steps: (1) transfer of phosphorus from the benzene solution to an aqueous medium by reaction with copper nitrate solution, (2) conversion of the copper phosphide to orthophosphate, and (3) determination of orthophosphate by either volumetric or gravimetric method.

An aliquot of the solution of phosphorus in benzene is transferred to a 250-ml. Erlenmeyer flask containing an excess of 25 per cent copper nitrate solution—i. e., about 20 grams of $Cu(NO_3)_2$ per gram of phosphorus. The phosphorus solution should be drawn into the transfer pipet by a vacuum pump, an aspirator bulb, or a safety pipet filler. The quantity of copper nitrate solution should be sufficient to show a distinct greenish tint in the solution after the copper phosphide precipitate settles.

The flask is stoppered and shaken vigorously at about 2-minute intervals for 10 to 15 minutes; the stoppers and sides of the flask are washed down with water, and the mixture is heated on a water bath to volatilize the benzene. A few glass beads are added to prevent bumping. About 5 ml. of concentrated nitric acid are added cautiously, and the heating is continued until the precipitated phosphides are dissolved. At this point, the clear solution is made up to 250 ml. with distilled water, and an aliquot, representing not more than 15 mg. of phosphorus, is transferred to a 500-ml., wide-mouthed Erlenmeyer flask. About 10 to 15 ml. of 60 per cent perchloric acid are added and the solution is digested on a hot plate. The heating should be so adjusted that the perchloric acid is seen to condense in the neck of the flask

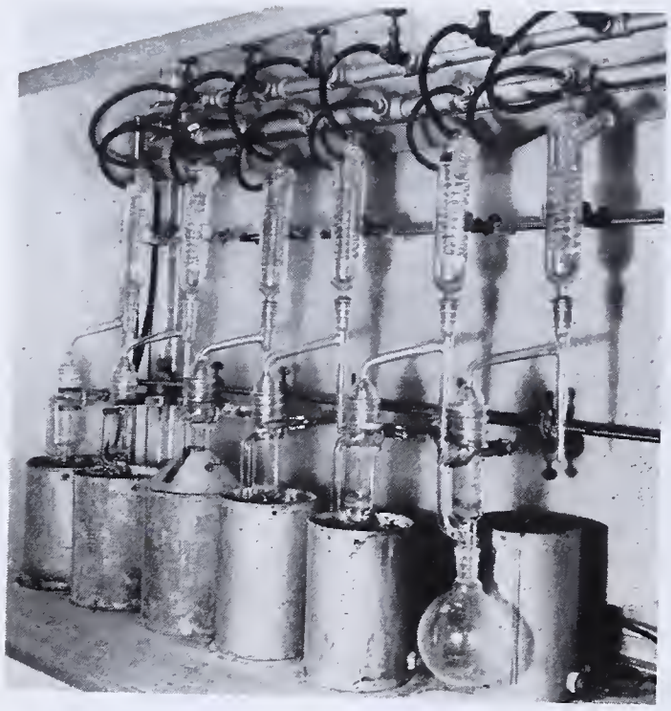

FIGURE 4. EXTRACTOR UNITS

Metal cans are electrically heated water baths. Conical cover on third bath from left aids in heating sintered-glass disk.

about 2.5 to 3.25 cm. (1 to 1.5 inches) below the top. Heating too vigorously will cause the acid to creep over the edge, while heating too gently will fail to oxidize the phosphorus completely. The heating is continued until the solution volume is 2 to 3 ml. The solution is then cooled and neutralized, and the orthophosphate content is determined by a standard method.

When the method given by Brown, Morgan, and Rushton was used by the analysts in the routine control laboratory, considerable difficulty was encountered in obtaining check results from aliquots of the same benzene solution. The time allowed for completion of the action of the initial addition of the nitric acid–bromine solution and the subsequent rate of boiling the mixture apparently influenced the degree of oxidation of the phosphorus and the hydrolysis of the resultant oxides. The use of perchloric acid after decomposition of the copper-phosphide precipitate gives uniformly reproducible results.

Table I shows the results obtained by two analysts using the modified procedure. Both analysts used aliquots of the same benzene solution of phosphorus.

TABLE I. ANALYSIS OF PHOSPHORUS

Analyst	Phosphorus
	%
A	98.8
	99.0
	99.0
B	99.1
	99.1
	99.0

The sum of the benzene-soluble phosphorus and benzene-insoluble residue seldom equals 100 per cent. The sample used to obtain data given in Table I contained 0.6 per cent benzene-insoluble material. The difference of 0.4 per cent between 100 per cent and the sum of the benzene-insoluble material and phosphorus covers the inaccuracies of the method and unaccounted for substances such as silicon tetrafluoride and other compounds of fluorine soluble in phosphorus and benzene.

Acknowledgment

The author wishes to acknowledge the work of other members of the TVA chemical engineering staff in the development of the procedures described in this paper.

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PRESENTED before the Division of Physical and Inorganic Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Oxidation of Graphite in the Analysis of Ferrous Metals

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IN THE determination of manganese and phosphorus in irons containing graphitic carbon it has been necessary to filter off the graphite after the sample is in solution. During solution water must be continually added to prevent the separation of gelatinous silica which would render the graphite filtration difficult. With many irons these operations may require an hour or more.

While perchloric acid will dissolve the sample rapidly, it oxidizes graphite only slightly. However, if 0.2 gram of sodium dichromate (or other soluble chromium salt) is added to the sample and the contents of the flask are fumed strongly, the graphite will be completely and rapidly oxidized. The chromium is subsequently volatilized quickly (*2*) by the addition of sodium chloride. A single determination for manganese can be made in 25 minutes, and 12 to 18 determinations in an hour.

The method can also be adapted to the determination of phosphorus with at least two advantages. The use of nitric acid in conjunction with the usual hydrochloric-perchloric acid mixture has been found to eliminate the loss of phosphorus as phosphorus hydrides, and the rapid oxidation of the graphite saves much time as compared with prior procedures.

Procedure for Manganese

To a 1.000-gram sample (0.5 gram for over 1.25 per cent of manganese) in a 500-ml. Erlenmeyer flask add 0.2 gram of solid sodium dichromate, 5 ml. of dilute hydrochloric acid (1 to 1), and 25 ml. of 72 per cent perchloric acid. Heat until dense white fumes issue from the mouth of the flask; then volatilize the chromium by adding 0.5 gram of sodium chloride at short intervals until the red fumes cease to be evolved upon the addition of the salt. Remove the flask from the hot plate, let it cool somewhat, wash down its neck and sides with water, and again heat until its contents fume. When the flask clears of white fumes (fumes will still form at the mouth), remove it from the hot plate.

Add 3 ml. of 85 per cent phosphoric acid, 10 ml. of 0.8 per cent silver nitrate solution, 100 ml. of warm water, and 2 ml. of 25 per cent ammonium persulfate solution. Boil for 2 to 3 minutes to decompose peroxides, add 8 ml. of 25 per cent ammonium per-

Table I. Comparison of Manganese Determinations by Different Methods

Sample	Manganese Found: A. S. T. M. method E30-39 (1)	Manganese Found: $HClO_4$–$Na_2Cr_2O_7$–NaCl method
	%	%
Bureau of Standards, 6d, 1.60% Mn, 2.69% C	1.60	1.60
Iron A, 4% C	0.77	0.79
Iron B, 4% C	1.10	1.12
Iron C, 4% C	0.87	0.88
Iron D, 4% C	0.15	0.14
Iron E, 2% C	0.24	0.23

Table II. Comparison of Phosphorus Determinations by Different Methods

Sample	Phosphorus Found: A. S. T. M. method E30-39 (1)	Phosphorus Found: $HClO_4$–$Na_2Cr_2O_7$–NaCl method
	%	%
Pig iron A, 4.5% C	0.205	0.206
Pig iron B, 4.5% C	0.198	0.197
Pig iron C, 4.5% C	0.177	0.174
Pig iron D, 4.5% C	0.073	0.072

sulfate solution, and boil for 30 seconds. Cool and titrate with arsenite solution (3.75 grams of sodium arsenite per liter), standardized against a standard steel or iron.

Procedure for Phosphorus

To a 1.000-gram sample in a 500-ml. Erlenmeyer flask add 0.2 gram of sodium dichromate, 10 ml. of dilute nitric acid (1 to 2), 5 ml. of dilute hydrochloric acid (1 to 1), and 25 ml. of 72 per cent perchloric acid. Heat until dense white fumes issue from the mouth of the flask and volatilize the chromium by adding 0.5-gram portions of sodium chloride as directed in the procedure for manganese. Remove the flask from the hot plate and allow it to cool. Add 40 ml. of water and 10 ml. of 20 per cent sodium hydrogen sulfite solution, twirl the flask, and add 10 ml. of dilute nitric acid (1 to 2). Boil for 5 minutes, cool to room temperature, add 50 ml. of ammonium molybdate solution (1), and let stand for 0.5 hour with occasional shaking. Finish as usual by filtering, dissolving the well-washed precipitate in excess alkali, and titrating the excess with standard nitric acid solution.

A standard iron of an analysis similar to the unknown sample should be carried through all the steps in order to standardize the nitric acid.

Caution

Perchloric acid must be considered a hazard and handled with caution at all times. If a bit of paper, oil, cloth, or other organic matter finds its way into fuming perchloric acid it may cause a disastrous explosion. So far the authors have encountered no difficulty when using these methods.

Conclusions

Chromic acid in fuming perchloric acid oxidizes graphite completely.

Nitric acid added at the beginning of a perchloric acid phosphorus determination prevents the loss of phosphorus as hydrides.

The solution of iron is hastened and the graphite is readily removed by the use of perchloric acid and a soluble chromium salt.

Application of these methods to the determination of manganese and phosphorus in irons containing graphite gives results agreeing with the older methods and effects a time saving of 40 to 50 minutes.

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A Simple Sintered-Glass Salt Bridge

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IN CERTAIN types of potentiometric measurements it is desirable to use a salt bridge of small cross section and low electrical resistance without the use of agar gels. The bridge of Irving and Smith (2) is advantageous in providing a direct liquid junction, but has a high electrical resistance due to its ground-glass joints; moreover, the type of construction requires tubing of relatively large diameter. Other types of bridges allowing direct liquid contact, such as that of Bright and Miller (1), often have the disadvantage of complexity of construction and tend towards high electrical resistance.

The sintered-glass bridge described here can be made of 3-mm. tubing if desired, is simple, has a low electrical resistance, and effectively eliminates siphon action. Thus it is unnecessary to maintain a constant level of solution throughout the system.

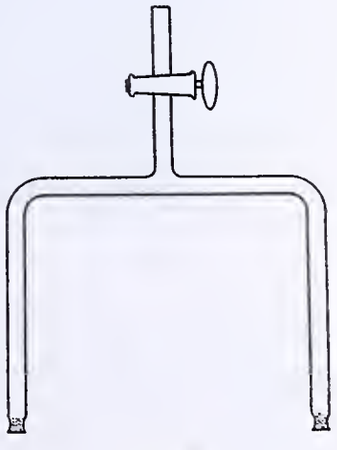

Figure 1

Method of Construction

Pyrex glass is ground to a fine powder in a porcelain mortar. The 3- to 10-mm. Pyrex tubing to be used is sealed at one end and filled to a length of a few centimeters with the powdered glass. The glass powder is warmed gently to allow expansion of the gases in it, and the glass is tapped to settle the powder in the end of the tube. The tubing is now heated with rotation, at some distance from the end, in a very small oxygen flame. As soon as the tubing begins to soften it is withdrawn rapidly from the flame and suction is applied with the mouth partially to collapse the tubing. The end of the tube is cut off, and the excess powdered glass is removed. The sintered-glass membrane can be tested by suction; it should be dense enough so that water can barely be sucked through with the mouth. With practice the membranes can easily be constructed; the main difficulty is in preventing complete fusion of the powdered glass. The bridge is completed as shown in Figure 1. Either one or both ends may be provided with the sintered-glass diaphragm, and the stopcock may be omitted.

The bridge is cleaned and filled by applying an aspirator or suction pump to the vertical arm, shutting off each end of the bridge in turn, if necessary, by means of a piece of rubber tubing provided with a clamp.

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(1) Bright, W. M., and Miller, E. L., Ind. Eng. Chem., Anal. Ed., 9, 346 (1937).
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Determination of the Aniline Point of Dark Petroleum Products

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THE aniline point (*1*) of a petroleum product is defined by the American Society for Testing Materials as the minimum equilibrium solution temperature for equal volumes of aniline and the petroleum product. The method of test (*1*) for determining the aniline point proposed by the A. S. T. M. depends upon the visual observation of turbidity in the mixture, and the test is intended for examination of oils not darker than No. 8 A. S. T. M. color (*1*). The aniline points of darker oils must therefore be determined by other means.

Methods for determining the aniline point of dark oils are described by Donn (*2*) and by van Wijk and Boelhouwer (*4*).

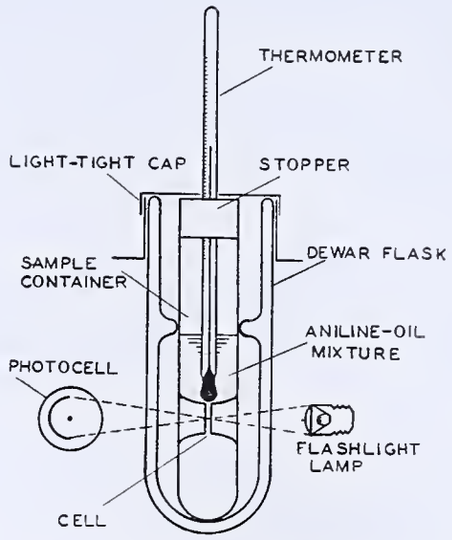

FIGURE 1. DIAGRAM OF MECHANICAL EQUIPMENT

Donn's procedure consists in determining the temperature at which a break occurs in the viscosity-temperature curve; this temperature is designated as the aniline point. The viscometer used by Donn could not be used for very dark oils. The aniline point measurement of very dark oils was restricted to an approximate method in which the occurrence of turbidity was observed in the 50/50 volume per cent mixture under a microscope.

Van Wijk and Boelhouwer (*4*) make use of the variation in transparency to infrared radiation of an equal volume mixture of oil and aniline. At the aniline point a marked change in the transparency is noted, owing to the appearance of a second liquid phase in the form of fine droplets.

The purpose of the present paper is to describe a simplified type of apparatus employing infrared radiation for detecting the point at which separation of the phases begins.

Description of Apparatus

MECHANICAL EQUIPMENT. A diagram of the arrangement of the essential parts of the mechanical equipment is shown in Figure 1. The sample container is a Pyrex test tube, approximately 25-mm. outside diameter, with depressions as shown to form a "cell" 1 to 2 mm. thick and about 10 mm. in diameter. Light from the 1.1-volt lamp passes through the cell and falls upon the gas-filled photocell, which has maximum sensitivity at 7500 Å. Substitution of the photocell for the thermopile used by van Wijk and Boelhouwer permits the use of an inexpensive microammeter as the indicating instrument.

The sample container is held in a Dewar flask and is stoppered with a rubber stopper holding a mercury-in-glass thermometer of suitable range. A light-tight cap covers the whole assembly in the box containing the complete apparatus. A photograph of the equipment is shown in Figure 2.

ELECTRICAL SYSTEM. A diagram of the electrical circuit is shown in Figure 3. The light from the lamp, T_3, controls the current flowing through the photocell, T_1. This current sets up a voltage across R_5 which is applied to the grid of the vacuum tube, T_2, to control the plate current through the meter, M. Any change in the amount of radiation to the photocell due to a change in the condition of the medium between the photocell and the lamp will be shown as a change in the reading of the meter. The initial reading of the meter is set by resistor R_2 with resistor R_3 limiting the maximum bucking current. The sensitivity of the system is varied by resistor R_1. Resistor R_4 limits the current in the lamp to the maximum safe value to obtain long life from the lamp.

Method of Operation

Twenty milliliters of the aniline-oil mixture are placed in the stoppered sample container. The stopper is fitted loosely, and the mixture is heated to a point well above the minimum equilibrium solution temperature. The heated mixture is then shaken thoroughly and the walls of the container are inspected visually to observe possible cloudiness. If a cloud is noted the sample is heated further and visual inspection is made again. A little experience enables the operator to determine the proper amount of heating in a single operation.

FIGURE 2. ANILINE POINT INDICATOR

The heated sample is placed in the Dewar flask at room temperature in such a position that the plane of the cell is normal to the incident radiation, the light-tight cap is placed in position, switches S_1 and S_2 are closed, resistance R_1 is set to maximum sensitivity, and R_2 is adjusted to bring the needle of the microammeter to a reading of approximately 90 per cent full scale deflection. Where temperature–meter deflection curves

are desired, the temperature indicated by the thermometer and the meter reading, at the instant a given temperature is indicated, is recorded at suitable intervals. The data are plotted as shown in Figure 4. The point at which phase separation begins is indicated by the sharp break in the curve.

Upon completion of the test, the contents of the sample container are poured out and the container is washed with a suitable solvent. In the case of very viscous samples of low aniline point it may be necessary to heat the tube in order to permit the sample to flow freely.

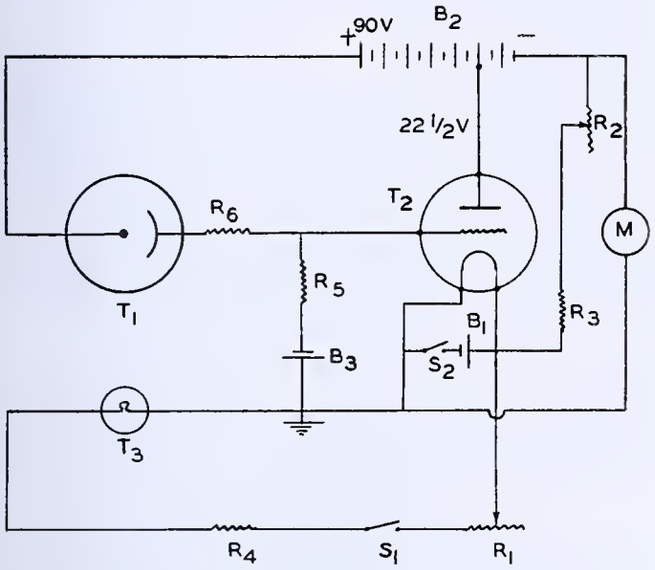

FIGURE 3. DIAGRAM OF ELECTRICAL CIRCUIT

B_1. 1.5-volt dry cell
B_2. 2–45 volt B batteries
B_3. 1-volt bias cell
M. 0–100 microampere meter, 3-inch face, 900 ohms
R_1. 6-ohm variable resistor, sensitivity control
R_2. 5000-ohm variable resistor, meter zero control
R_3. 1500-ohm fixed resistor
R_4. 1.7-ohm fixed resistor
R_5. 300-megohm fixed resistor
R_6. 2-megohm fixed resistor
S_1. Single-pole single-throw toggle switch
S_2. Single-pole single-throw toggle switch
T_1. RCA Type 868 photocell
T_2. Type 1G4G vacuum tube
T_3. Type 112 1.1-volt, 0.3-watt Pencell lamp, Mazda

Where no record of the temperature–meter deflection curve is to be kept, the operator simply observes the point at which the needle of the meter begins to move sharply. With the proper sensitivity setting and optimum film thickness, this point is always well defined.

The time required for a determination is dependent upon the difference between the aniline point of the oil being tested and the surrounding temperature—approximately room temperature. For example, if a material having an aniline point only slightly above room temperature is heated to a high temperature, say 80° C., at the beginning of the test, a half hour or more may be required for the solution to reach the aniline point. On the other hand, an oil having an aniline point above approximately 50° C. will reach its minimum equilibrium solution temperature in a few minutes, even if heated considerably above its aniline point. Average time for a test, including loading the sample container, heating, and cooling, is between 15 and 20 minutes.

Calibration

By following the procedure outlined above, the temperature of the mixture drops at the natural cooling rate. The thermometer bulb is placed about 5 mm. above the center of the cell. Under these conditions, the temperature indicated by the thermometer is somewhat higher than that in the cell, depending upon the rate at which the temperature is falling.

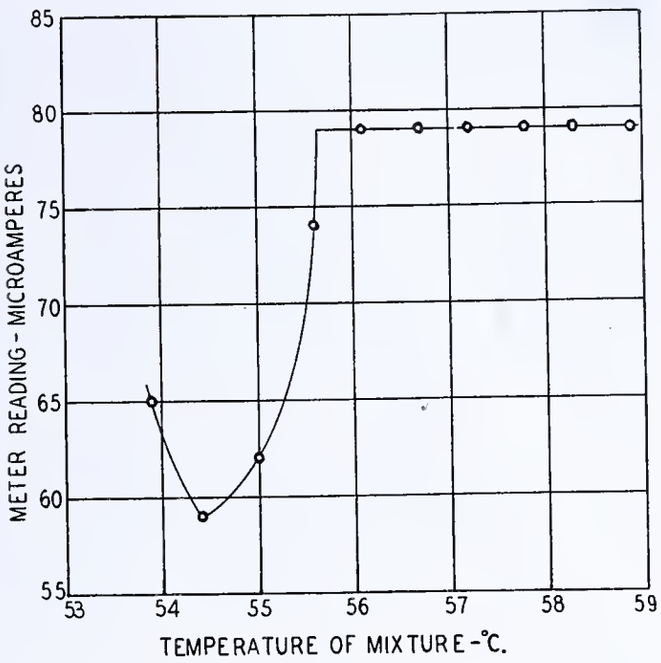

FIGURE 4. ANILINE POINT OF TRANSPARENT OIL
Comparison of infrared radiation and visual methods

The absence of stirring also tends to give a value for the aniline point that will be somewhat higher than that determined by the visual method. The difference in the aniline point as determined by the above procedure and by the visual methods increases with the value for the aniline point. This makes it necessary to establish a correction curve (Figure 5) by determining aniline points by the two procedures on a series of transparent samples covering a wide range of aniline points above room temperature. The reason for the rapid increase in the value of ΔT (infrared aniline point – visual aniline point) with aniline point is that the temperature-time curve follows

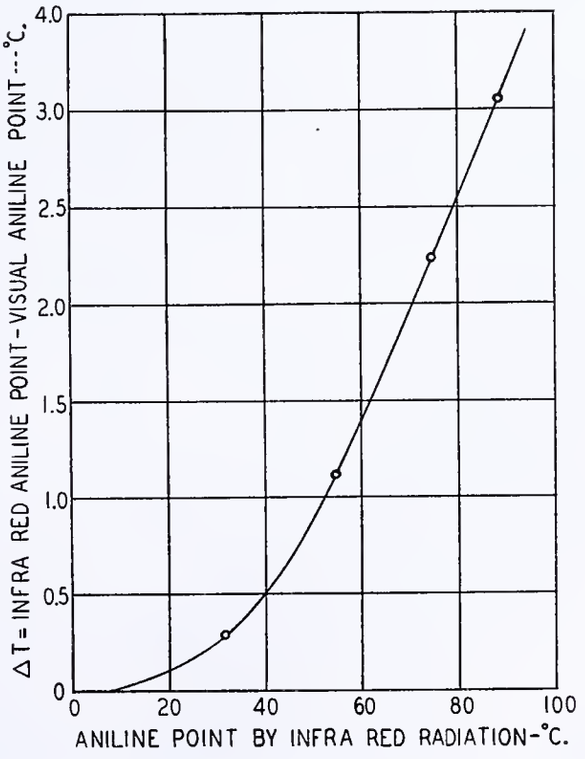

FIGURE 5. CORRECTION CURVE FOR INFRARED ANILINE POINT INDICATOR

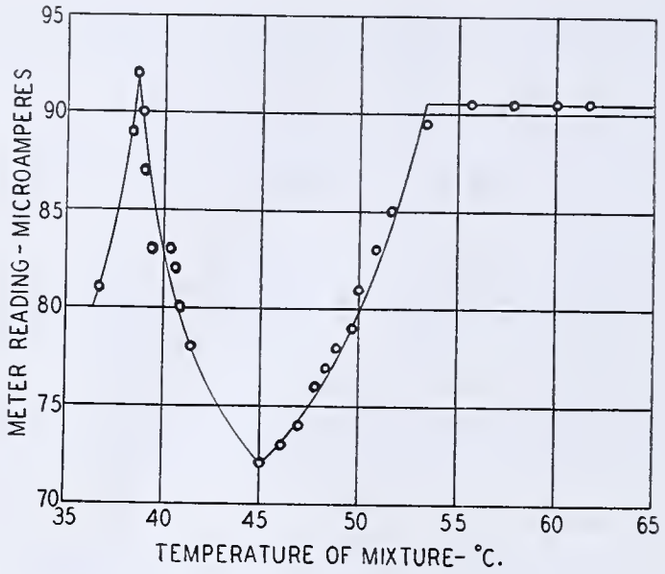

FIGURE 6. ANILINE POINT OF CRACKED TAR DETERMINED BY INFRARED RADIATION

an exponential law. The rate at which the temperature of the liquid is falling is as follows:

Temperature, ° C.	$\frac{d\theta}{dt}$ deg./min.
90	4.8
75	2.8
60	1.3
45	0.6

θ = temperature
t = time

Results

The apparatus and procedure described here have given satisfactory results on a wide variety of stocks, including straight-run residue, cracked tars, extracts from solvent-refining operations, and black fuel oils.

Figure 4 is the temperature–meter deflection curve for a transparent mixture of aniline and a blend of a highly paraffinic lubricating oil and a low aniline point extract. The point at which phase separation took place, as indicated by the meter, was 55.6° C. From Figure 5 the value for the temperature correction, ΔT, is 1.1° C.; and the aniline point by the infrared method is 54.5° C. The value obtained by the visual method was 54.4° C. Comparison of the results obtained with transparent oils tested by both the visual and infrared methods indicates that when the correction from Figure 5 is applied, the two methods check within 1° C.

Application of the correction for cooling obtained from Figure 5 increases the accuracy of the present infrared method over that of van Wijk and Boelhouwer, who obtain agreement within 2° C. on tests of duplicate samples.

The curve shown in Figure 6 was obtained on a black cracked tar. The aniline point after applying the correction, ΔT, is 51.7° C.

In general, the form of the curves is erratic after phase separation has begun. The left side of Figures 4 and 6 clearly shows this. The erratic behavior is due to variations in the state of the dispersion of the separated aniline and to their effect on the amount of transmitted radiation. Secondary effects enter as other solubility phenomena appear. For example, in the case of the cracked tar–aniline solution, a point is reached where the transmission of radiation is greater than it was when the solution was above its separating temperature. This is probably due to the formation of large globules of aniline which transmit a wide band of radiation extending into the visible region.

Mixtures were found in a few cases, such as those described by van Wijk and Boelhouwer, wherein the aniline-oil mixture was more opaque above the solution temperature than after separation of the phases took place. The temperature–meter deflection curves for these mixtures break sharply upward at the aniline point in a direction opposite to that shown in Figures 4 and 6.

The aniline point of an oil whose solution temperature with aniline is below room temperature is determined by blending the oil with a hydrocarbon diluent of higher aniline point. One such method (*3*) involves the use of a standard diluent such as a narrow boiling petroleum fraction of 60° C. (140° F.) aniline point and approximately 130 average molecular weight. A 50/50 per cent by volume mixture is made of the sample and the diluent. The aniline point of this blend is then determined. The aniline point of the sample is read from a reference curve showing the relation between the aniline points of oils and blends of these with the diluent. The reference curve is established by the visual method on a series of transparent oils covering a wide range of solution temperatures.

Acknowledgment

Thanks are due to R. A. Krause for his valuable assistance in developing the electrical system.

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(4) Van Wijk, W. R., and Boelhouwer, J. W. M., *J. Inst. Petroleum Tech.*, **24**, 598 (1938).

Thermoelectric Effects in Photometry

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A PHOTOMETER employing the barrier-layer type of cells for measuring light intensity can find many applications both in industry and other fields, particularly as an aid to routine testing. Since most light sources have a variable intensity it is necessary to compensate for this, and it is usually done by a second photocell connected in opposition to the first across a suitable galvanometer. As pointed out by Müller (*3*) the compensating device can be either optical or some form of resistance bridge. If the latter device is used it may be subject to considerable errors unless certain precautions are taken.

Consider the circuit suggested by Wilcox (*4*); a balance is obtained by varying resistors R_1 and R_2 (Figure 1). In practically every case the metal of the sliding contact and that of the resistance wire will be different and will therefore be a potential source of a thermoelectric e. m. f. On varying the resistance, the friction of the contact arm on the wire causes a rise in temperature and the Seebeck effect at once becomes considerable, the magnitude depending upon the metals of the resistance and speed of variation. For certain types of photometry this effect will probably be negligible, but for

Table I. Thermoelectric Effects

			Sample A		
$R_2' - p$	510	510	510	510	510
$R_2'' - p$	500	500	500	500	500
Mean	505	505	505	505	505
			Sample B		
$R_2' - p$	435	430	430	430	430
$R_2'' - p$	435	435	435	435	435
Mean	435	432	432	432	432
			Sample C		
$R_2' - p$	360	365	370	365	365
$R_2'' - p$	325	320	320	325	325
Mean	342	342	345	345	345

more accurate work where small differences in intensity are to be measured it becomes important and must be eliminated.

Gall (*2*) in a comprehensive survey of potentiometers discusses the effects of thermoelectric e. m. f.'s when measuring fractions of a μv and states "the galvanometer itself may have dissimilar metal junctions and must therefore be kept free from temperature changes. Brass terminals are not advisable and copper should be used throughout." It is not always easy or practicable to make a circuit of one metal only, but in the case of the photometer resistance bridge it has been found possible to eliminate thermoelectric e. m. f.'s.

The thermoelectric effects caused by friction can be eliminated by using a resistance made of copper throughout. If the resistor is to be small and compact its resistance must be low (<50 ohms), since it must be wound with copper wire. This small variable resistance may form only part of one arm of the bridge, R_2, the remainder being made up with a fixed resistance, R_1 also being a fixed resistance. Thus the range of intensity variation that can be covered will be small (since R_2 is variable only over a small range); but when it is necessary to measure very small differences in intensity only a small range is required as a rule.

It is possible that thermoelectric e. m. f.'s may be set up because different parts of the circuit are at different temperatures. This may sometimes be avoided by shielding and lagging, though it is more satisfactory to employ a method of reversals. The following treatment demonstrates how stray e. m. f.'s may be eliminated. (It is assumed that the reversing switch is made of solid copper throughout, so that no Seebeck effects are produced when it is operated.)

Using Müller's (*3*) notation, when the bridge is balanced,

$$ig = 0 = \frac{E_2 - E_1}{r_g} = \frac{kIR_2 - kITR_1}{r_g}$$

But when a small thermoelectric e. m. f. is produced the above relation is modified since E_2 becomes $E_2' \pm e$, where e is the algebraic sum of all such e. m. f.'s and varies with time. E_2' is the photovoltage.

$$ig = \frac{E_2' \pm e - E'}{r_g} = \frac{kIR_2' + e - kIR_1T}{r_g} \quad (1)$$

$$= \frac{kIR_2'' - e - kIR_1T}{r_g} \quad (2)$$

where R' and R_2'' are values of R_2 before and after reversal, since e changes sign with respect to E_1 and E_2.

Adding (1) and (2)

$$T = \frac{R_2' + R_2''}{2R_1}$$

$$T = K\frac{R_2' + R_2''}{2} \quad (3)$$

Therefore $T \propto$ mean of readings before and after reversal. Thus by varying R_2 until a balance is obtained and then reversing the polarity of the cells and rebalancing, the mean of the two readings of R_2 gives the correct result independent of thermoelectric e. m. f.'s; since over the time of reversal it can safely be assumed that the temperatures of the various parts of the circuits have remained unaltered.

Equation 3 has been tested experimentally using a reversing switch designed for the purpose. This switch has been described elsewhere (*1*) and provides convenience of operation (the potentials of both cells are reversed in one movement); absence of thermoelectric effects in the switch itself on operation (this is attained by making every metal part of pure copper); and negligible contact resistance; and it is self-cleaning.

Using an all-copper resistance to form the variable portion of R_2 as indicated above, the results presented in Table I were obtained which demonstrate the validity of Equation 3 and the necessity under certain circumstances for a method of reversals.

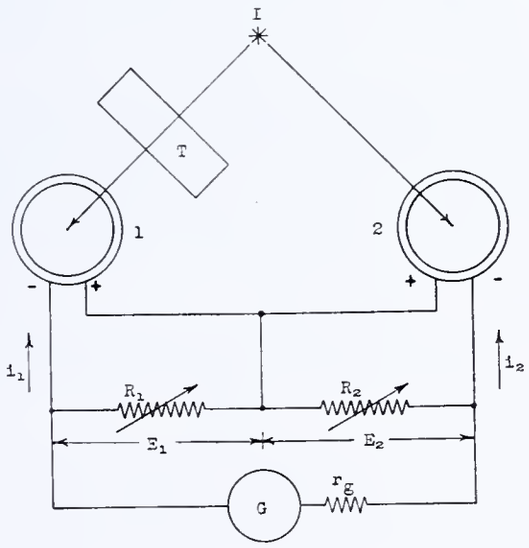

Figure 1. Balanced Circuit Possessing Compensation for Source Fluctuations

On warming one of the copper-nickel junctions in the circuit with the fingers a thermoelectric potential is set up, giving a large difference between R_2' and R_2''. Over a period of time this difference decreases as the junction cools to the temperature of its surroundings.

Sample *B*, after creating a thermoelectric potential, gave the following values.

$R_2' - p$	530	540	500	480	470
$R'' - p$	340	320	370	390	400
Mean	435	430	435	435	435

p is the value of the fraction of R_2 which remains fixed; the figures given above are values of the variable portion of R_2 in arbitrary units.

Acknowledgment

The author is indebted to the directors of Messrs. Courtaulds, Ltd., for permission to publish this paper.

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Viscosity of Chocolate

Development of Standard Method

JOSEPH STANLEY

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The theory and technique of the MacMichael viscometer applied to determine the viscosity of chocolate have been examined. A modified Couette equation holds dimensionally for the MacMichael instrument and is

$$\eta = \frac{\theta G d^4 (1/a^2 - 1/b^2)}{128 L \Omega l}$$

It is advisable to express viscosities in degrees MacMichael because of practical, commercial, and logical considerations. A standard technique is described to embrace the entire range of chocolate viscosities on the same basis.

The factors influencing viscosity are discussed—cocoa butter, lecithin, moisture, air, fineness, and temperature. It is shown that besides saving cocoa butter, counteracting moisture, and stabilizing chocolate, lecithin protects colloidal dispersion, especially when the chocolate is in the melted, heated, or overheated state.

Rheograms of chocolate show the influence of various factors on plastometric properties—for example, how the "yield stress" represents a numerical measure of "gumminess", and how the "body" represents a numerical measure of the equally elusive "coverage" of chocolate.

WITH the growing industrial importance of viscosity in chocolate technology numerous methods, most of them empirical, have been developed. The technical, analytical, and commercial importance of viscosity is now well established (*20*). The MacMichael test (*10*) and the expression of chocolate viscosity in degrees MacMichael are so prevalent that any method studied must be based on this instrument. However, no single set of MacMichael conditions will cover the entire range of chocolate viscosities with accuracy, and this study was undertaken to develop a standard method and standard mode of expression. Final results could easily have been expressed in terms of absolute viscosity, but no such recommendation is made, since there is no logical reason why MacMichael degrees should not be used, and any change from the existing mode of expression would meet with resistance from manufacturing and purchasing personnel.

Any chocolate is essentially a dispersion of finely comminuted cocoa matter, sugar, and sometimes milk solids in cocoa butter. The viscosity of the resulting melted chocolate depends on such things as kind, time, and temperature of processing; particle size, shape, and kind; and percentages of cocoa butter, lecithin, moisture, and air. Other factors have been amply covered (*18*, *19*, *21*). How the viscosity of a particular dark chocolate varies with progressive additions of cocoa butter and lecithin is depicted in Figure 1. *A* shows the optimal percentage of lecithin (0.35 per cent) causing the same viscosity reduction as *B*, which corresponds to about 8 per cent of cocoa butter. This curve also shows that addition of lecithin above the optimal percentage will cause either no change or an increase in viscosity. We can thus use this change in viscosity on adding, say, 0.1 per cent of lecithin, to an unknown sample of chocolate as a presumptive test for the presence of a viscosity-reducing agent.

Bases of Various Viscosity Methods

The methods and instruments used to determine the viscosity of chocolate can be divided into several classes. Any method that gives viscosity at a standard temperature and rate of shear should be satisfactory (*12*).

The efflux method due to Poiseuille is well known. The viscosity is defined by the familiar equation:

$$\eta = \frac{\pi P R^4 t}{8 V h} \qquad (1)$$

The use of Pochettino's (*14*) method has been suggested, since most chocolates are rather viscous. In this process we observe the time of ascent to a measured height of the sample in a capillary tube under vacuum. Since during this test the time and height are variable, the Pochettino equation is derived from Poiseuille's equation by appropriate integration between desired limits:

$$\eta = \frac{P R^2 t}{4 h^2} \qquad (2)$$

When the viscosity approaches the highest ranges and the sample is more or less of a semisolid, instruments based on Segel's (*17*) method can be used:

$$\eta = \frac{A}{2\pi B v} \ln \frac{R_2}{R_1} \qquad (3)$$

The annular space of thickness $(R_2 - R_1)$ between concentric cylinders is filled with a height, B, of sample, and the relative velocity, v, is measured when the inner cylinder is allowed to slide under the influence of force A.

In Europe instruments based on Stokes' law are popular in the chocolate industry. Stokes' equation is

$$\eta = \frac{2}{9} g U^2 \frac{d_1 - d_2}{v} \qquad (4)$$

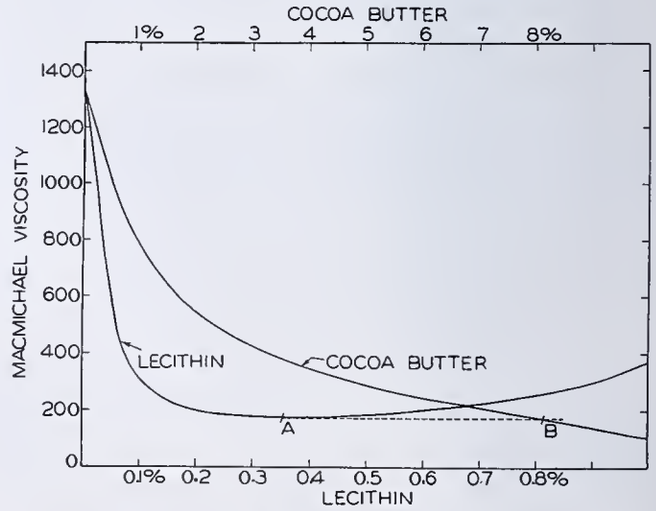


FIGURE 1. RELATIVE REDUCTIONS IN VISCOSITY OF A DARK CHOCOLATE

A. Optimal percentage of lecithin
B. Saving in cocoa butter

In Stokes' method we observe the velocity, v, of a small metallic sphere of density d_1 and radius U, falling under the influence of gravity in a liquid of density d_2. In the case of opaque liquids the descent may be followed by x-ray cinematography. Recently an automatic Stokes instrument has been offered for sale in England under the name of the "ball and bucket viscometer" (*1*). Similar methods are used in France and Germany.

Derivation of MacMichael Equation

The MacMichael viscosity method is widely used in the chocolate industry of the United States and Canada. However, the lack of standardized technique results in confusion when the figures of different observers are compared. Since the MacMichael method is proposed as standard the basis of this method will be investigated here.

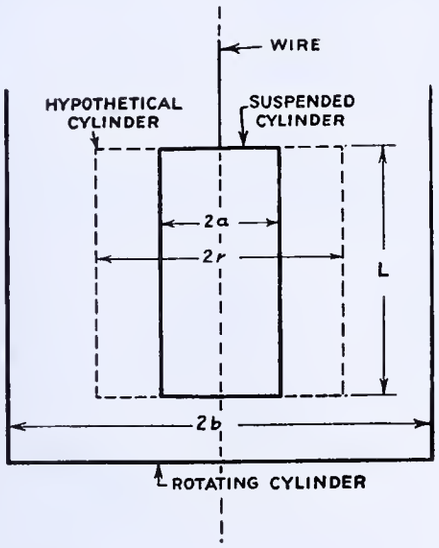


FIGURE 2. DIAGRAMMATIC SECTION OF MACMICHAEL ROTATING CYLINDER

We observe the angular torsion, θ, of a wire of length l, when one end is fixed and the other is attached to a cylindrical bob immersed in the melted chocolate placed in a rotating cup.

Margules (*2*, *11*) has calculated the viscous moment, M, at the lateral surface of a cylindrical bob of radius a and height L, when suspended in an outer cylinder of radius b, which contains the sample and rotates at angular velocity Ω. Figure 2 shows these two concentric cylinders diagrammatically. Since the outer cylinder (cup) rotates at constant angular velocity Ω, the chocolate may be thought to rotate in concentric layers ranging in linear velocity from 0 at the surface of the inner cylinder (bob) to $b\Omega$ at the surface of the outer cylinder. If we consider an intermediate hypothetical cylinder of the same height as the bob, but of radius r in the chocolate, and assume the angular velocity in its surface to be ω, the linear velocity will increase at the rate of $rd\omega/dr$ as we proceed to the surface of the outer cylinder. The force due to the viscosity of the chocolate on the lateral surface of the hypothetical cylinder is

$$F = \eta r \frac{d\omega}{dr} 2\pi rL \tag{5}$$

and as this has an arm, r, the moment is

$$M = 2\pi\eta Lr^3 \frac{d\omega}{dr} \tag{6}$$

By integration between the indicated limits we get

$$\frac{1}{M}\int_0^{\Omega} d\omega = \frac{1}{2\pi\eta L}\int_a^b \frac{dr}{r^3} \tag{7}$$

or

$$M = \frac{4\pi\eta L\Omega}{1/a^2 - 1/b^2} \tag{8}$$

Equation 8 defines the viscous moment, but does not relate it to the angular torsion of the MacMichael wire. To calculate this we proceed as follows (*7*):

The angular torsion, θ, in the wire of polar moment of inertia I_P, length l, modulus of rigidity G, due to moment M, is

$$\theta = \frac{Ml}{I_P G} \tag{9}$$

and since

$$I_P = \frac{\pi d^4}{32} \tag{10}$$

we get by substitution in Equation 8:

$$\theta = \frac{128\,\eta L\Omega l}{Gd^4(1/a^2 - 1/b^2)} \tag{11}$$

Equation 11 contains all the variables and is fundamental to this discussion. However, if b is very large in comparison with a, and remembering that $D = 2a$, we get a simplification which is sometimes useful:

$$\theta = \frac{32\eta LD^2\Omega l}{Gd^4} \tag{12}$$

Since the MacMichael instrument is a Couette type of viscometer, the modified Couette Equation 11 may be expected to apply. The author has investigated the dimensional obedience to Equation 11 by varying the diameter of the wire, d, the length of immersion of inner cylinder, L, etc. The analysis is based upon assumed equal modulus of rigidity and absence of set of the torsion wire. While the moduli have not been measured directly, the obedience to Equation 11 indicates that the postulate is correct. In addition he has encountered no trouble due to set, owing to the relatively large diameters of the wires used.

NOMENCLATURE

- a. Radius of inner cylindrical bob, cm.
- A. Force, dyne
- b. Radius of holding cup, cm.
- B. Height of sample, cm.
- d. Diameter of wire, cm.
- d_1. Density of small sphere, grams per cc.
- d_2. Density of fluid, grams per cc.
- D. $2a$, diameter of inner cylindrical bob, cm.
- η. Viscosity, poise, dyne seconds per sq. cm.
- F. Viscous force in surface of bob, dyne
- g. Acceleration due to gravity, cm. per sec.^{2}
- G. Modulus of rigidity of wire's metal, dyne per sq. cm.
- h. Height, cm.
- I_P. Polar moment of inertia of wire's cross section, cm.^{4}
- l. Length of wire, cm.
- L. Length of inner cylindrical bob, cm.
- M. Moment, dyne cm.
- P. Pressure, dyne per sq. cm.
- r. Radius of hypothetical cylindrical bob, cm.
- R. Radius of tube, cm.
- R_1. Radius of outer cylinder in Equation 3, cm.
- R_2. Radius of inner cylinder in Equation 3, cm.
- t. Time, seconds
- θ. Angular torsion of wire, radians
- U. Radius of small sphere, cm.
- v. Velocity, cm. per second
- V. Volume, cc.
- ω. Angular velocity, considered at radius r, rad. per second
- Ω. Angular velocity of MacMichael cup, rad. per second

Viscosity Tests on Typical Samples

We see from Equation 11 that the torsional displacement or MacMichael reading is directly proportional to viscosity, length of bob's immersion, and square of bob's diameter, and is inversely proportional to the fourth power of the wire's diameter.

In the chocolate industry B. & S. wire gages Nos. 22 to 32 are used, with cylindrical bobs of 1- or 2-cm. diameter at 2- or 4-cm. height.

Specific details on the manipulation of the MacMichael instrument have been given elsewhere (*5*, *6*, *9*, *15*, *21*). The range of viscosities encountered is between a fraction of a poise and several thousand poises, and no single set of MacMichael conditions can accurately cover this entire range.

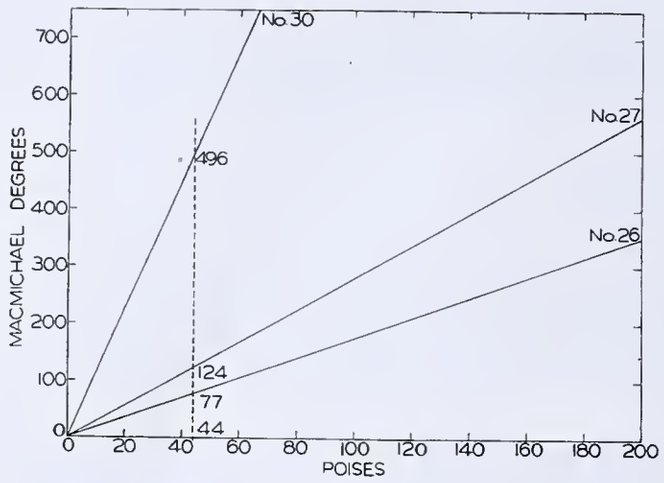


FIGURE 3. CONVERSION OF STANDARD MACMICHAEL VISCOSITY TO POISES

The MacMichael conditions used by most are No. 27 wire, 2-cm. diameter bob, 4-cm. height, 20 r. p. m., 37.8° C. (100° F.), and 7-cm. diameter cup. Since these conditions do not cover the range it is necessary to change the size of the wire, the diameter of the bob, or the immersed length of the bob. In Figure 3 is shown the poise viscosity corresponding to MacMichael readings using wires of various sizes. This graph is also useful in converting readings from one wire to the other (dotted line).

To determine what conditions are best ten samples of chocolates were tested under many conditions designed to cover the whole range. The entire instrument and appliances were kept in a closet at 37.8° C. (100° F.). Five hundred grams of each sample were slowly melted in an oven at 65.6° C. (150° F.), but the temperature of the chocolate never exceeded 43.3° C. (110° F.).

To indicate briefly the conditions which are varied the notation 27, 4, 2 is used to indicate size of wire, length of immersion, and diameter of bob.

The viscosities obtained are summarized in Table I. Table II gives properties of torsion wires commonly used to test chocolate products.

Table I indicates that 3 wires and 2 bobs can cover the entire range of chocolate viscosities. Since 27, 4, 2 was chosen as the standard, the other conditions must be converted to this standard. Table III gives factors for conversion to standard conditions, calculated according to Equation 11, and also shows the range of viscosities that can be covered with each set of conditions.

A No. 30 wire is extremely convenient in the ice cream coating industry to avoid a double standard. Calculations according to Equation 12 show that readings on this wire divided by 4 give standard MacMichael degrees on a No. 27 wire.

Before the standard viscosities of the ten samples are calculated it is necessary to determine which conditions are convertible. Table IV shows that conversion from a 1-cm. bob to the standard 2-cm. bob is justified.

TABLE II. DIAMETER, SAFE LIMIT, AND STRAIN POINT OF TORSION WIRES COMMONLY USED TO TEST CHOCOLATE PRODUCTS

B. & S. No.	Diameter *Inch*	Safe Limit	Strain Point
22	0.02535	100	125
23	0.02257	150	175
24	0.02010	200	225
25	0.01790	250	275
26	0.01594	350	375
27	0.01419	350	375
28	0.01264	350	375
29	0.01126	350	375
30	0.01003	375	400
31	0.00893	375	400
32	0.00795	375	400

TABLE III. VISCOSITY RANGES AND FACTORS FOR CONVERSION TO STANDARD CONDITIONS

Conditions	Range	Factor
27, 4, 2	50– 300	1.000
27, 4, 1	300–1200	4.000
26, 4, 2	100– 450	1.592
26, 4, 1	500–2000	6.370
30, 4, 2	0– 50	0.250

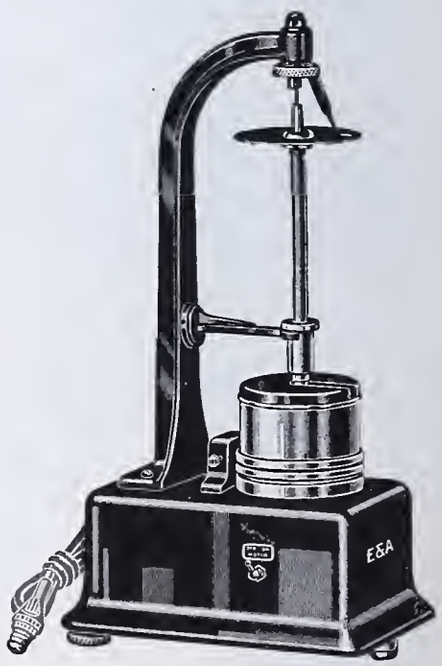
TABLE IV. RELATION OF READING ON 1-CM. TO 2-CM. BOB AND CONVERSION TO STANDARD VISCOSITY

Sample No.	Kind	Viscosity 27, 4, 2	Viscosity 27, 4, 1	Conversion to Standard Factor 4.000
2	Liquor	95	24	96
4	Light sweet	214	51	204
7	Milk chocolate	268	65	260
9	Ice cream coating	12	3	12
10	Ice cream coating	5	1	4
		26, 4, 2	26, 4, 1	To 2-Cm. Diameter
3	Dark	232	57	228
5	Milk	222	54	216
8	Buttermilk	275	70	280

TABLE I. MACMICHAEL DEFLECTIONS OF TYPICAL CHOCOLATE PRODUCTS AT 100° F.

Sample No.		27, 4, 2	27, 4, 1	26, 4, 2	26, 4, 1	30, 4, 2	27, 2, 2	26, 2, 2	26, 2, 1
1	Cocoa butter	1	..	...	..	4 4[a]	...	...	..
2	Liquor	95 107[a]	24	59	..	...	51	...	..
3	Dark	..	93[a]	232	57 58[a]	...	199	129	..
4	Light sweet	214	51 50[a]	138	..	...	122	...	..
5	Milk chocolate, 12% milk solids	..	..	222	54 52[a]	...	191	123	..
6	Milk chocolate, 18% milk solids	..	251	...	161 159[a]	...	...	350	78
7	Milk chocolate, 0.25% lecithin, 18% milk solids	268	65 64[a]	168	..	...	146	...	..
8	Buttermilk chocolate, 0.15% lecithin	..	..	275	70 69[a]	...	248	160	..
9	Ice cream coating, 55% cocoa butter	12	3	8	..	43 58[a]	7	...	..
10	Ice cream coating, 0.25% lecithin, 65% cocoa butter	5	1	3.5	..	20 23[a]	3	...	..

[a] Small 3-cm. cup used.



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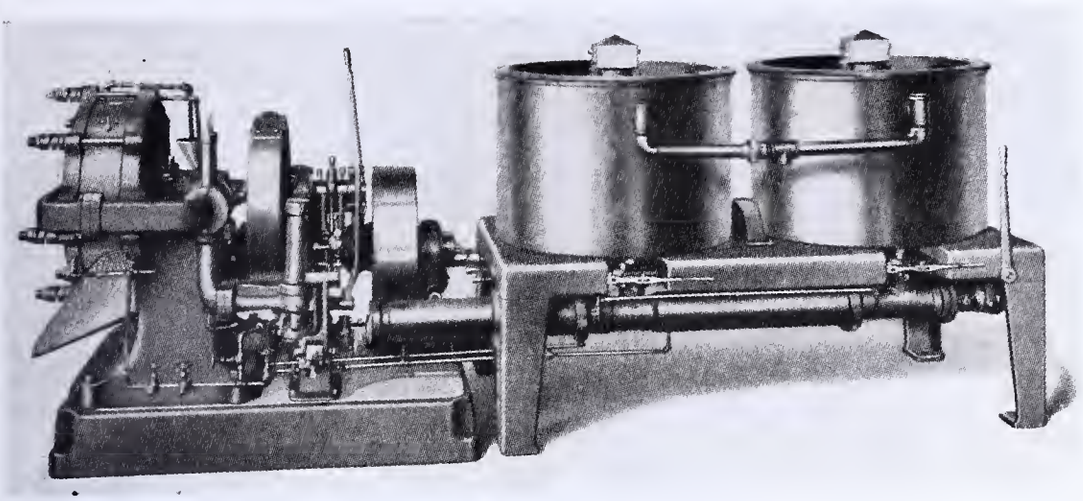
MACMICHAEL VISCOMETER USED TO DETERMINE VISCOSITY OF CHOCOLATE



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BAUSMAN DISK CHOCOLATE REFINER

full 4-cm. immersion to standardize this error, particularly when bobs of diameters greater than 1 cm. are used.

Frequently only a small amount of sample is available for the viscosity test. The author investigated the possibility of using the small 3-cm. cup for this test, since only about 30 grams are needed. As shown in Table VII, the 3-cm. cup will give almost exactly the same results as a 7-cm. cup, provided a 1-cm. bob is used. The 2-cm. bob should never be used with a 3-cm. cup, as this gives high results (Table I, samples 2, 9, and 10). Equation 11 clearly shows how the diameter of the outer cup influences the torsional displacement.

Table VIII gives the standard viscosities, either read directly or calculated from the indicated conditions which are known to give an accurate conversion.

TABLE V. EFFECT OF WIRE DIAMETER ON MACMICHAEL DEFLECTION

Sample No.	Kind	Viscosity No. 27	Viscosity No. 26	Conversion to No. 27, Factor 1.592
2	Liquor	95	59	94
3	Dark	93	57	91
4	Light sweet	214	138	220
6	Milk	251	161	257
7	Milk	268	168	268
			No. 30	Factor 0.250
1	Cocoa butter	1	4	1
9	Ice cream coating	12	43	11
10	Ice cream coating	5	20	5

TABLE VI. COMPARISON OF MACMICHAEL DEFLECTIONS AT 4- TO 2-CM. HEIGHT

Sample No.	Kind	Viscosity 4-cm. height	Viscosity 2-cm. height	Conversion to 4-Cm., Factor 2
2	Liquor	95	51	102
3	Dark	232	129	258
4	Light sweet	214	122	244
5	Milk chocolate	222	123	246
6	Milk chocolate	161	78	156 (1-cm. diameter bob)
7	Milk chocolate	268	146	292
8	Buttermilk chocolate	275	160	320

TABLE VII. COMPARISON OF MACMICHAEL DEFLECTIONS USING 7- AND 3-CM. CUPS, 1-CM. DIAMETER BOB

Sample No.	Kind	Viscosity, 7-Cm. Cup	Viscosity, 3-Cm. Cup
3	Dark	57	58
4	Light sweet	51	50
5	Milk chocolate	54	52
6	Milk chocolate	161	159
7	Milk chocolate	65	64
8	Buttermilk chocolate	70	69

Table V proves that the conversion from a No. 26 or No. 30 wire to the standard No. 27 can be made with sufficient accuracy.

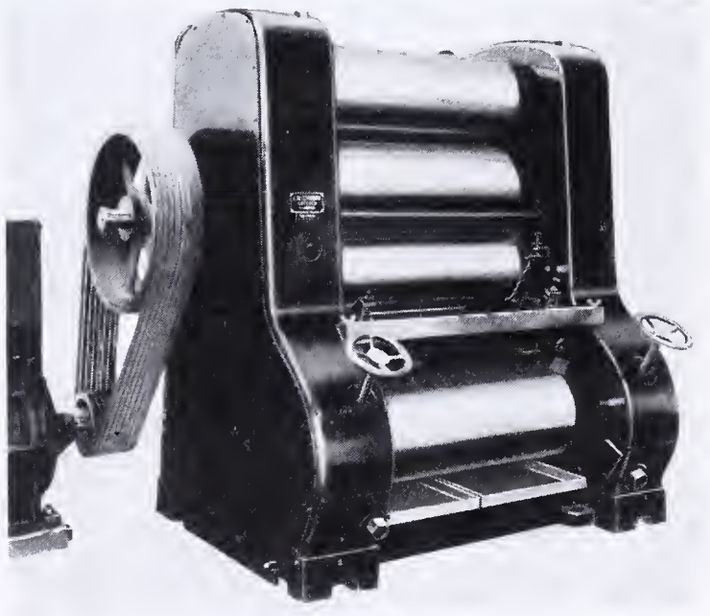
Table VI gives the results obtained using a 4-cm. height in comparison with a 2-cm. height, other conditions remaining the same. In almost every case the result obtained by doubling the reading at 2-cm. height is much higher than the reading at the regular 4-cm. height. In theory the bob should rest on an air bubble at its concave bottom, thus avoiding any contact of the bottom with the sample. Owing to the high viscosities the bottom is actually wetted, giving rise to high results. Accordingly all readings should be made with the

TABLE VIII. STANDARD VISCOSITIES OF ALL CHOCOLATE SAMPLES

Sample No.	Kind	Standard Viscosity	How Obtained
1	Cocoa butter	1	30, 4, 2
2	Liquor	95	Direct
3	Dark chocolate	370	26, 4, 2
4	Light sweet	214	Direct
5	Milk chocolate	354	26, 4, 2
6	Milk chocolate	1004	27, 4, 1
7	Milk chocolate	268	Direct
8	Buttermilk chocolate	438	26, 4, 2
9	Ice cream coating	12	30, 4, 2
10	Ice cream coating	5	30, 4, 2

Effect of Heat and Temperature

The effect of heat and temperature on chocolate must be studied before a rational standard viscosity method can be



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HIGH-SPEED 5-ROLL MILL USED TO REFINE CHOCOLATE

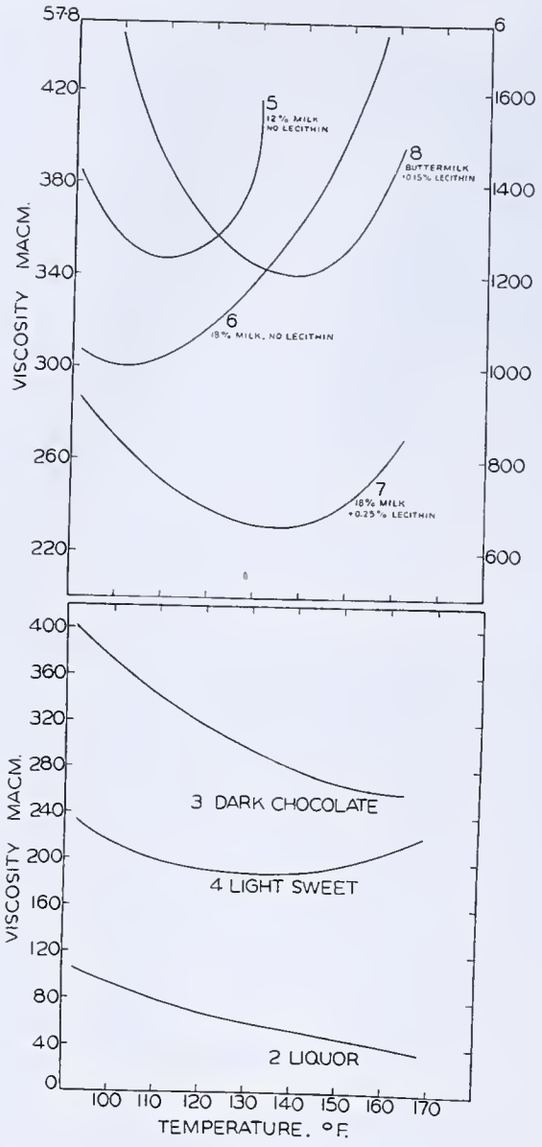


FIGURE 4. EFFECT OF RISING TEMPERATURE ON VISCOSITY OF TYPICAL CHOCOLATES

TABLE IX. EFFECT OF DRASTIC HEAT ON VISCOSITY OF CHOCOLATE PRODUCTS

Sample No.	Kind	Viscosity before Heating	Viscosity after Heating
1	Cocoa butter	1	1
2	Liquor	95	90
3	Dark chocolate	370	357
4	Light sweet	214	220
5	Milk chocolate, 12% milk solids	354	489
6	Milk chocolate, 18% milk solids, no lecithin	1004	1830
7	Milk chocolate, 18% milk solids, with lecithin	268	364
8	Buttermilk chocolate	438	789
9	Ice cream coating, 55% cocoa butter	12	12
10	Ice cream coating, 65% cocoa butter	5	5

TABLE X. EFFECT OF RISING TEMPERATURE ON VISCOSITY OF CHOCOLATE

Sample No.	Kind	95 °F	100 °F.	105 °F.	110 °F.	120 °F.	130 °F.	140 °F.	150 °F.	160 °F.
2	Liquor	101	95	88	80	69	61	54	48	42
3	Dark chocolate	390	370	356	340	318	298	281	265	249
4	Light sweet	227	214	206	199	189	188	190	197	209
5	Milk chocolate, 12% milk solids	370	354	349	347	355	414	..	..	..
6	Milk chocolate, 18% milk solids, no lecithin	1015	1004	1004	1025	1100	1195	1335	1530	..
7	Milk chocolate, 18% milk solids, with lecithin	281	268	260	251	239	231	232	242	264
8	Buttermilk chocolate	463	438	413	392	359	344	340	355	390

TABLE XI. INVERSION TEMPERATURES OF CHOCOLATE, SHOWING BENEFICIAL EFFECT OF LECITHIN

Sample No	Kind	Inversion Temperature ° F.	Sample No.	Kind	Inversion Temperature ° F.
2	Liquor	Above 160	6	Milk chocolate, 18% milk solids, no lecithin	102
3	Dark chocolate	Above 160	7	Milk chocolate 18% milk solids, with lecithin	135
4	Light sweet	135	8	Buttermilk chocolate with lecithin	138
5	Milk chocolate, 12% milk solids	110			

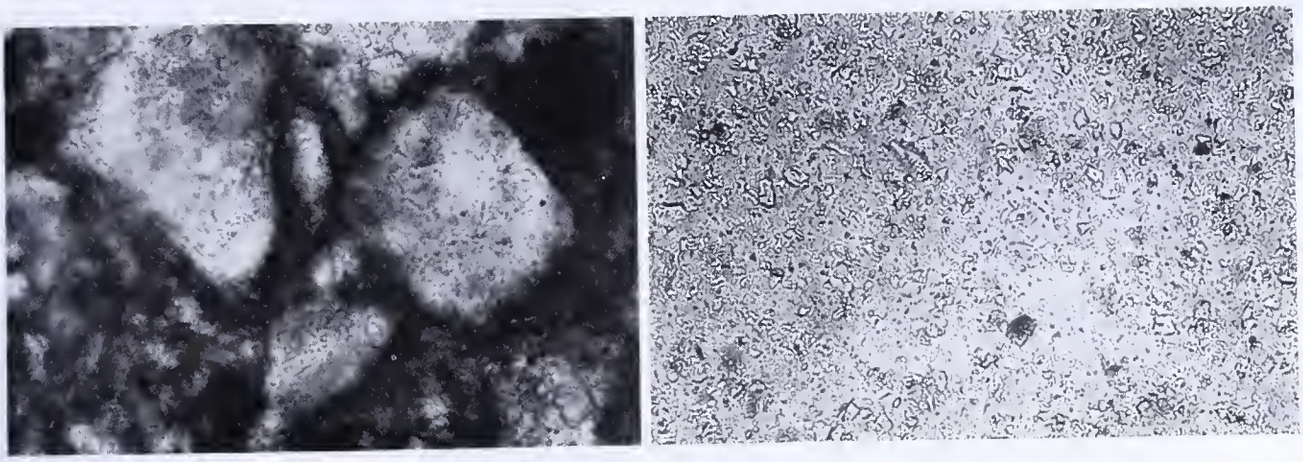
formulated. Dark chocolates will tend to get thinner and milk chocolates thicker when subjected to excessive heat. To show this effect a 454-gram (1-pound) sample of each was heated in an aluminum double boiler for 15 minutes. The water boiled briskly, the temperature of the chocolate rose to about 93.3° C. (200° F.), and it was stirred only occasionally. The chocolate was in almost every case completely ruined, particularly the milk chocolates (Table IX). However, in actual manufacturing practice vigorous agitation greatly modifies the harmful influence of heat.

In order to show the effect of temperature on chocolate, viscosities were determined at intervals between 35° and 71.1° C. (95° and 160° F.). Within this range the dark chocolates thin down continuously, although much above 71.1° C. (160° F.) they too thicken up considerably. The light sweet and milk chocolates thin down and reach a minimum (the inversion point), after which further increases in temperature produce a rapid thickening effect. The viscosity was determined immediately after bringing the melted sample to the indicated temperature in a viscosity cup.

The effect of temperature on chocolate is most important when preparing the sample for test. The results of Table X, plotted in Figure 4, have many technical implications as well. The curves show what happens when a chocolate is heated at random, and prove the necessity of careful melting. For example, samples 6 and 7 are the same except that No. 7 contains 0.25 per cent of added lecithin (Table XI). It is seen at once that lecithin has a striking effect in raising the inversion temperature, in this particular case 18.3° C. (33° F.). The lecithin helps to maintain the colloidal dispersion of the milk solids particles during severe heat treatment of milk chocolate.

Table XII shows the relative increases in viscosity due to moisture in two portions of the same chocolate, reduced to the same viscosity with 0.3 per cent of lecithin and with 6 per cent of cocoa butter. This table indicates the necessity of avoiding moisture when preparing a sample for test, and shows the protective effect of lecithin in counteracting the thickening effects of moisture.

Numerous other factors influence the viscosity of chocolate—for example, agitation, particularly in the absence of lecithin, will reduce the viscosity of some chocolates (Table XIII). The viscosity may even fall when the solidified chocolate is stored for long periods of time. The harmful fall of viscosity on storage (*21*) is done away with by "stabilizing" the chocolate with lecithin. For technical reasons some chocolates are aerated, with a consequent sharp increase in viscosity. Excessive agitation must therefore be avoided



Courtesy, J. M. Lehmann Co., Inc.

PHOTOMICROGRAPH OF MILK CHOCOLATE (× 200)
Left. Coarse particles before refining
Right. Same milk chocolate after refining

TABLE XII. EFFECT OF MOISTURE ON VISCOSITY OF CHOCOLATE WITH AND WITHOUT LECITHIN

Moisture %	Chocolate with 0.3% Lecithin	Chocolate with 6% Cocoa Butter
0.50	112	112
0.75	130	132
1.00	143	150
1.25	187	195
1.50	230	238
1.75	250	290
2.00	330	440

TABLE XIII. EFFECT OF AGITATION ON VISCOSITY OF AERATED CHOCOLATE

Agitation *Hours*	Viscosity	Agitation *Hours*	Viscosity
0	665	3	452
1	572	4	410
2	503	6	368

when preparing the sample for viscosity test, although for ordinary chocolates the little agitation necessary to carry out a viscosity test will not appreciably change the viscosity.

Chocolate is essentially a dispersion of finely ground edible particles in a fat. The colloidal phenomena attending such a system have been amply discussed (*18*, *19*, *20*). The most important factors influencing the viscosity of chocolate are cocoa butter and lecithin (*20*), as shown in Figure 1. The finer the grind the higher will be the viscosity or plasticity (*16*). In unstabilized products mixing has the effect of gradually reducing viscosity because of the gradual completion in the wetting of the disperse phase.

Viscosity Procedure

Bearing in mind previous work (*5*, *6*, *9*, *15*, *21*) and the results of this investigation, we are now ready to formulate the details for determining the viscosity of chocolate using the MacMichael instrument. First of all, there is no reason why the conditions now used by most cannot be made standard: No. 27 wire, 2-cm. diameter bob, 4-cm. height, 20 r. p. m., 37.8° C. (100° F.), and 7-cm. diameter cup. If the viscosity cannot be accurately determined using these conditions because it falls without the range, suitable conditions are chosen from Table III, and the viscosity is reduced to standard conditions by the appropriate factor.

Place the viscometer and appliances in a closet fitted with a light and thermometer, so that everything is maintained at a temperature of 37.8° C. (100° F.). Melt a 500-gram sample in an aluminum or glass beaker in an oven kept at 65.6° C. (150° F.). In the time that it takes to melt the sample the temperature of the chocolate should not rise above 43.3° C. (110° F.) and is usually less. When melted, stir gently until uniform, avoiding excessive agitation. Transfer to a 7-cm. cup, adjust to 37.8° C. (100° F.), and read the viscosity at 4-cm. height. If little sample is available use a 3-cm. cup, with a 1-cm. bob. Convert to standard viscosity by using the appropriate factor from Table III.

In case of very heavy chocolates, canalization may take place around the 4-cm. height mark, resulting in an effective decrease in depth of immersion. In this case the highest reading is taken, since on prolonged rotation the canalization increases and the reading decreases.

For compound or imitation chocolate coatings in which the melting point of the fat may be close to or above 37.8° C. (100° F.) a temperature of 48.9° or 54.4° C. (120° or 130° F.) is used in the test, and an appropriate note made in the report.

Rheological Investigation

All the important factors in MacMichael Equation 11 have now been examined, save the speed of rotation. Bingham and Mooney (*16*) distinguish between the viscosity of true liquids and the rigidity of plastic materials. To give a complete representation of the viscometric or plastometric behavior of a material a rheological diagram must be plotted, giving the rate of shear against stress. It has been shown by Perrott and Thiessen (*13*) that the speed of rotation of the MacMichael instrument is proportional to the rate of shear, while the deflection is proportional to stress. Table XIV gives the standard MacMichael deflections at various speeds of rotation. Figures 5 and 6 are plots of r. p. m. against deflections, or rheological diagrams. At the same time the

TABLE XIV. MACMICHAEL DEFLECTIONS OF CHOCOLATE PRODUCTS AT VARIOUS SPEEDS OF ROTATION

Sample No.	Kind	Specific Gravity, 100°/100° F.	MacMichael Deflections 10 r. p. m.	20 r. p. m.	30 r. p. m.	40 r. p. m.
1	Cocoa butter	0.899	0.5	1.0	1.25	2.0
2	Liquor	1.104	71	95	120	143
3	Dark chocolate	1.238	295	370	448	498
4	Light sweet	1.259	158	214	270	314
5	Milk chocolate	1.217	252	354	443	540
6	Milk chocolate	1.242	825	1004	1140	1225
7	Milk chocolate	1.242	209	268	326	383
8	Buttermilk chocolate	1.258	321	438	555	665
9	Ice cream coating	1.110	7	12	16.5	21.5
10	Ice cream coating	1.042	3	5	7	9

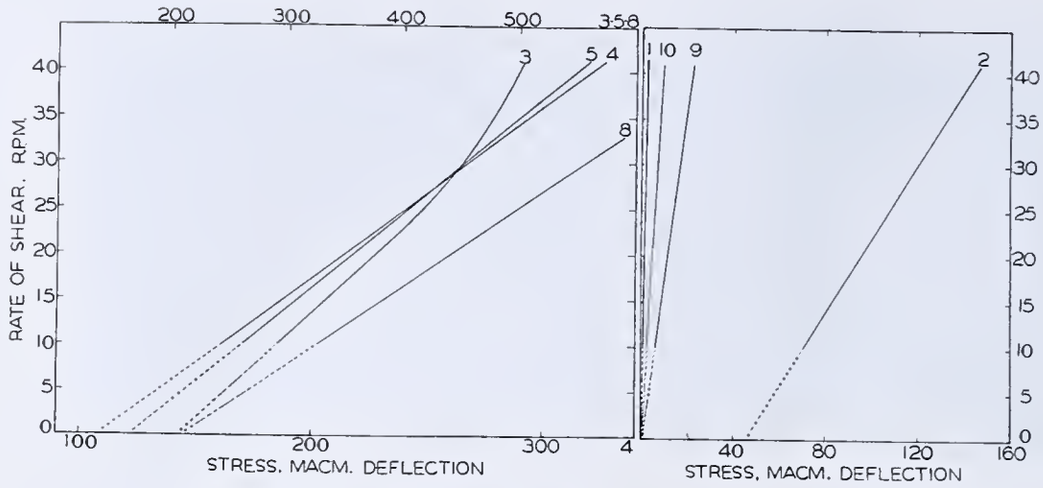


FIGURE 5. RHEOGRAMS OF TYPICAL CHOCOLATE PRODUCTS

1. Cocoa butter
2. Liquor
3. Dark chocolate
4. Light sweet chocolate
5. Milk chocolate
8. Buttermilk chocolate
9, 10. Ice cream coating

In Table XV are given in comparison to the standard viscosity, the yield stress, the rigidity at 10 and 40 r. p. m., and the "body". The quotient of yield stress to standard viscosity is taken as a measure of the body of a chocolate. Thus a chocolate may be low in viscosity but high in body, or vice versa.

A complete plastometric analysis of chocolate will include the standard viscosity, the yield stress, the body, and the rigidities at low and high rates of shear (*12*). These facts are secured from the rheograms (Figures 5 and 6). The actual rheological curvature also gives important information.

specific gravity of each sample is given at 37.8°/37.8° C. (100°/100° F.), since this is an important factor in the plastic behavior of chocolate.

When consistent units are employed in plotting rheological diagrams (*8*) the ratio of stress to rate of shear at any point is numerically equal to the absolute viscosity. In the case of so-called true fluids or Newtonian fluids the rheological curve is a straight line passing through the origin. Generally the curves of plastic or non-Newtonian materials are of variable curvature, usually approaching the origin asymptotically. Following Bingham's (*3, 4*) treatment such curves may be extrapolated to the stress axis. This intercept is called the yield stress and is a measure of the "stay-putted-ness" or gumminess of a plastic material.

Figures 5 and 6 indicate that the yield stresses are roughly dependent on viscosity, and that the rheological curves do not deviate much from linearity around 20 r. p. m., which proves that the selected speed of rotation is sufficiently high.

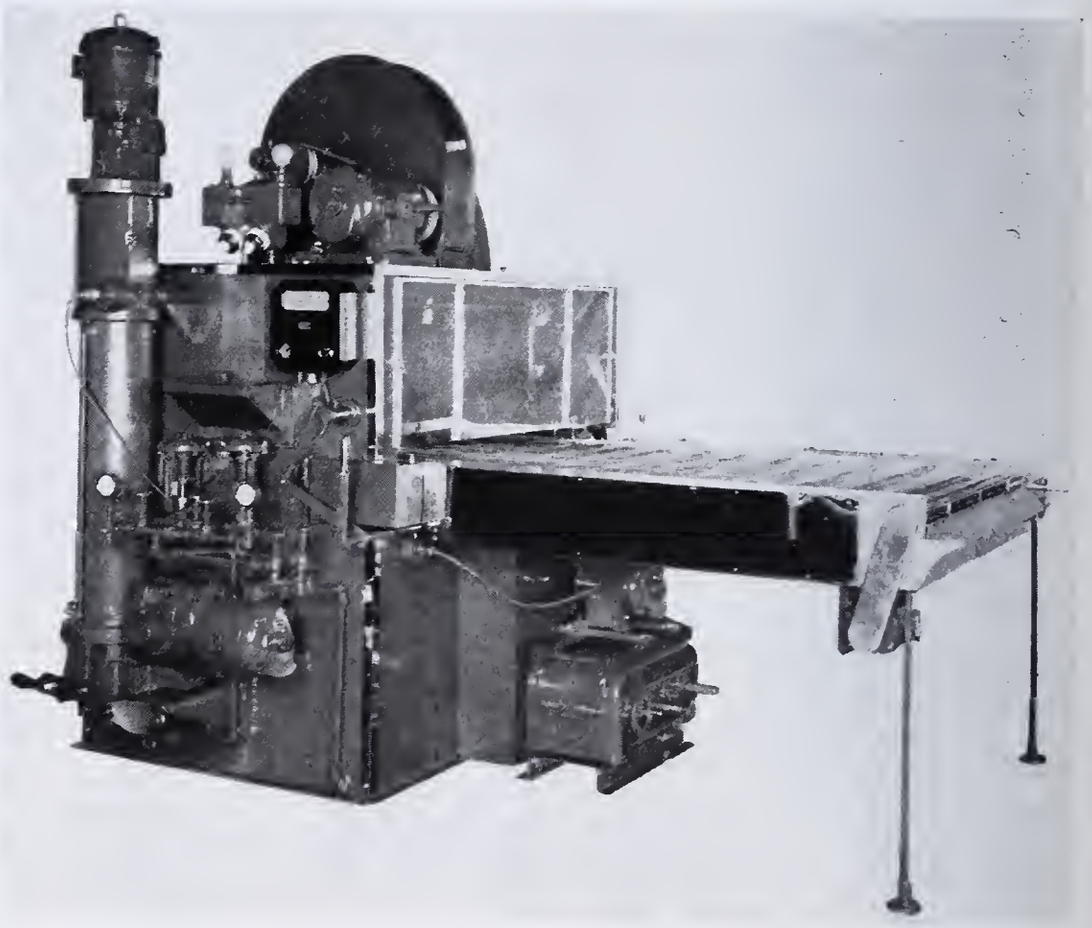
Following the recommendation of Perrott and Thiessen (*13*) the relative rigidities at low and high rates of shear are calculated from the formula

$$R = t(D_1 - D_0) \quad (13)$$

in which R = relative rigidity, t = period of rotation in seconds, D_1 = deflection at a certain r. p. m., and D_0 = deflection at 0 r. p. m. (yield stress).

TABLE XV. PLASTOMETRIC CONSTANTS OF CHOCOLATE SAMPLES

Sample No.	Kind	Standard Viscosity	Yield Stress	Rigidity 10 r. p. m.	Rigidity 40 r. p. m.	Body
1	Cocoa butter	1	0.25	1.5	2.6	25.0
2	Liquor	95	45.5	153	146	47.9
3	Dark chocolate	370	203	552	443	54.4
4	Light sweet	214	108	300	309	50.4
5	Milk chocolate	354	162	540	567	45.5
6	Milk chocolate	1004	650	1050	863	64.8
7	Milk chocolate	268	150	344	350	56.0
8	Buttermilk chocolate	438	209	672	684	47.4
9	Ice cream coating	12	2	30	29	16.7
10	Ice cream coating	5	1	12	12	20.0



Courtesy, National Equipment Co.

MODERN 50-INCH CHOCOLATE ENROBER

For example, in the case of curve 6, Figure 6, representing a sample of milk chocolate without lecithin, we have a condition of lower viscosity at higher stresses known as high structural viscosity. This rheological curve bends to the left, and the relative rigidities vary greatly. It shows a poor colloidal condition akin to an imperfect dispersion like mayonnaise, whose emulsion frequently breaks by simple transportation in a freightcar. The addition of 0.25 per cent of lecithin converts this milk chocolate into a perfectly dispersed colloid showing almost Newtonian characteristics as depicted by curve 7. Sample 3, a dark chocolate without lecithin, also displays a rheological curve which bends to the left, and has varying rigidities. This is probably due to its unusual fineness or special heat treatment in processing.

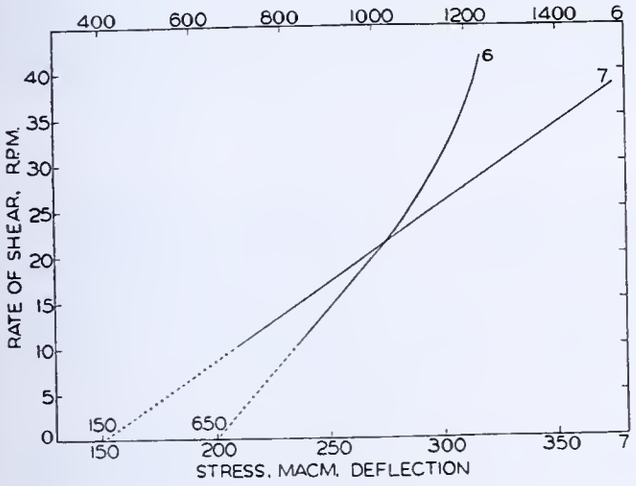


FIGURE 6. RHEOGRAM OF MILK CHOCOLATE
With (7) and without (6) lecithin, showing high structural viscosity in absence of lecithin

Table XV shows that the liquor has practically the same body as the buttermilk, despite the great difference in viscosity between these two samples. The body is as important a factor as the viscosity when considering the covering power of the chocolate. Indeed, we know from actual experience that despite its lower viscosity the liquor will show a covering power comparable to buttermilk chocolate of much higher viscosity.

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Continuous Water Remover

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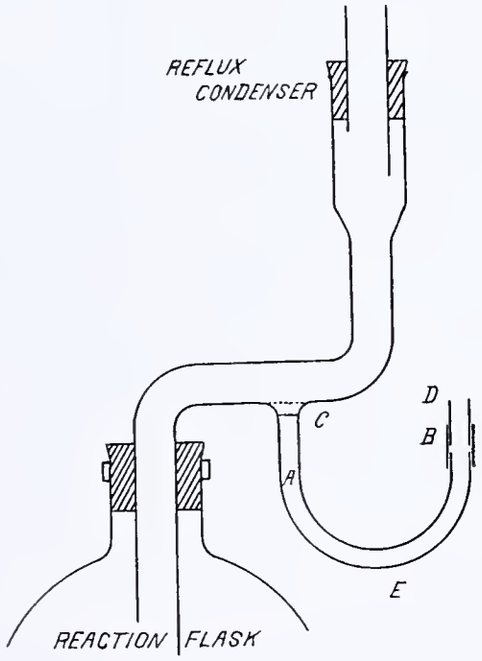
IN THE preparation of isoamyl ether from isoamyl alcohol by the use of concentrated sulfuric acid, it was deemed advisable to effect a continuous dehydration of the reaction mixture. Inquiries were made among other chemists with the disclosure that none was familiar with a water remover as simple in operation as the one described here. No particular claim for originality is made, but to the author's knowledge this particular modification is new.



The apparatus is simple and easy to construct, requires no attention during operation, and may be used in many other reactions besides distillations involving the removal of water.

The side arm, *A*, was filled to about point *C* with water at the start of the refluxing. The returning reflux liquids became entrapped in *A* and the water portion sank to the bottom. The excess alcohol overflowed back into the reaction flask. The water, on accumulation in *A*, overflowed from opening *D* and dripped off the side arm at *E*.

At *B* the side arm was made of two sections connected by a short length of rubber tubing. This made it possible to lengthen or shorten the overflow tube with a consequent raising or lowering of the alcohol-water interface at *C*. In this way only a small quantity of alcohol was entrained at *C* at any time.

Analyses of Sodium Meta-, Pyro-, and Orthophosphates

With Some Annotations on Methods

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IN A previous paper (*6*) the authors described the determination of meta-, pyro-, and orthophosphoric acids in mixtures by means of a series of three acidimetric titrations in which the acids are considered, with respect to one atom of phosphorus, as mono-, di-, and tribasic. The end points were defined in terms of colorimetric pH. The present paper outlines the application of these pH titrations to the assay of sodium phosphates and compares the method with a number of other modes of attack, in an endeavor to rationalize some of the larger discrepancies of analytical data shown by various test procedures.

In using the colorimetric pH titrations the same considerations (*6*) which applied to the polyphosphoric acids apply to the polyphosphates. The method determines the amount of phosphorus pentoxide associated with one, two, and three molecules of sodium oxide or water, which are reported as meta-, pyro-, and orthophosphate, respectively, but it does not distinguish between a polyphosphate and a mixture of meta- and pyrophosphate of equivalent proportions.

Determination by pH Titrations

The description of the analytical procedure for sodium phosphates is restricted, in so far as practicable, to an outline, since the theory, technique, limitations, and accuracy as well as many of the operations and solutions are the same as given in the earlier paper (*6*). The procedure has been in constant use in this laboratory for more than four years and has proved to be of much value in research and control work.

Reference should have been made in the earlier paper to prior workers who employed titration with alkali to the tribasic end point using excess silver nitrate, in particular to Balareff (*3*) who projected its application to mixtures of the three acids. The titration has been recurrently described without coming into general use. As indicator, lacmoid was used by Balareff, phenolphthalein in the presence of sodium acetate by Wilkie (*27*), methyl red by Moerck and Hughes (*17*) and by Sanfourche and Focet (*22*), and bromothymol blue by Simmich (*23*). The potential high accuracy of this titration is not realized, however, when used, as customary, in conjunction with the less satisfactory methyl orange and phenolphthalein titrations to the mono- and dibasic end points. The accuracy of these end points is greatly enhanced by definition in terms of colorimetric pH and by recognizing the differences between the equivalence points of the pyro- and orthophosphates.

Apparatus and Solutions

The method depends upon titrations with 0.1409 *N* sodium hydroxide solution (1 cc. = 0.01 gram of phosphorus pentoxide) changed from 0.1408 *N* (*6*) to agree with the 1940 atomic weight of phosphorus. The solution must be essentially carbonate-free and protected at all times against atmospheric carbon dioxide. The solution is standardized against benzoic acid, using standard (sample 39) and procedure of the National Bureau of Standards. When so standardized, the mean phosphorus pentoxide content of Sörensen grade primary potassium orthophosphate, dried at 105° C. and titrated directly to the silver nitrate-methyl red end point, was found to be 52.17 per cent, against 52.16 per cent by theory, with an average deviation from mean of 0.02 per cent phosphorus pentoxide in 10 titrations.

In addition to the items described in the earlier paper (*6*), sulfuric or nitric acid, roughly 0.5 *N*, is necessary. Either acid at 0.1409 *N* is useful in some modifications of the general method. The acid is standardized by titrating a measured amount with the 0.1409 *N* sodium hydroxide using phenolphthalein indicator.

The distilled water used in the titrations and in preparing the standard solutions should be vigorously aerated to remove carbon dioxide. Not more than 0.05 cc. of the 0.1409 *N* alkali should be required to bring 100 cc. of the aerated water to pH 8.8.

Procedure

The procedure given below is a general one for water-soluble sodium or potassium phosphates. Because carbonate may be present in the more alkaline phosphates, treatment to remove interference from this source is necessary. The removal is accomplished by aeration of an acidified solution of the sample; boiling is inadmissible because of rapid hydration to orthophosphate.

PREPARATION OF SOLUTION. Transfer a weighed sample containing about 1.6 grams of phosphorus pentoxide to a 100-cc. volumetric flask. Dissolve the sample in an excess of 0.5 *N* sulfuric or nitric acid, measured from a buret, together with sufficient water to give a volume of 50 to 70 cc. An excess of 3 to 5 cc., as shown by a drop of 0.4 per cent bromocresol green indicator, is suitable. Fit the flask with a 2-hole rubber stopper with one short tube for connection to an aspirator and one capillary tube drawn out to a very fine tip reaching nearly to the bottom of the flask. Pull air through the acidified solution under reduced pressure (less than 100 mm. of mercury) for 5 minutes to remove carbon dioxide. After dilution to the mark and mixing, transfer three 25-cc. aliquots, containing about 0.4 gram of phosphorus pentoxide, to each of three Almquist titrating flasks.

BROMOCRESOL GREEN TITRATION. Titrate one aliquot with standard alkali to the monobasic end point as described in the prior paper (*6*).

METHYL RED TITRATION. To the above titrated solution at the monobasic end point, add silver nitrate in excess and titrate to the tribasic end point with a standard alkali as described in the prior paper. Add water as desired to improve sedimentation. At the end point, a red color does not return upon further addition of silver nitrate but the addition of one drop of 0.1409 *N* acid should bring back a red or orange-red color which is not removed by vigorous agitation. The red color will sometimes slowly reappear upon standing, owing to hydration of meta- or pyrophosphate of the sample, but acidity from this source obviously should not be included in the titration.

THYMOL BLUE TITRATION. To another aliquot add 20 grams of sodium nitrate and titrate with standard alkali to the dibasic end point as described in the prior paper, substituting oleo red B indicator if required. A sodium nitrate blank, found by adjusting 100 cc. of water to pH 8.8, adding 20 grams of sodium nitrate, and titrating to pH 8.8, must always be subtracted from this titration.

METHYL RED TITRATION FOR TOTAL PHOSPHORUS. To the third aliquot add 7 cc. of nitric acid, specific gravity 1.42, and a few glass beads and dilute to 100 cc. Boil for 15 minutes to convert to orthophosphate. Cool, add one drop of bromocresol green indicator, and nearly neutralize with clarified 50 per cent sodium hydroxide solution. Cool again, add 0.5 cc. of 0.4 per cent bromocresol green solution, and accurately adjust with 0.1409 *N* alkali to pH 4.6 at 100-cc. volume. Add 25 cc. of 0.85 *N* neutral silver nitrate solution and 0.5 cc. of 0.2 per cent methyl red indicator, then titrate with 0.1409 *N* alkali as before until the red color of the indicator is just discharged, the solution then becoming greenish yellow in color.

Acid Equivalent Titration. Transfer the same amount of 0.5 N acid as used in preparing the sample solution to a 100-cc. volumetric flask and dilute to the mark. Titrate a 25-cc. aliquot with 0.1409 N sodium hydroxide using phenolphthalein indicator.

Calculations. Using the nomograph (*6*, Figure 3), determine the pH values to be used as end points for the phosphate mixture under test. Correct the titration volumes using these end points. Using these corrected volumes and with the titrations in order of their description designated as BCG, MR, TB, MRT, and AE, calculate the composition.

MRT × 0.005 = grams of total P_2O_5 ($o + p + m$)
(TB − BCG) × 0.01 = grams of P_2O_5 ($o + p$)
[MR − (TB − BCG)] × 0.01 = grams of P_2O_5 (o)
(AE − BCG) × 0.004367 = grams of alkalinity in terms of Na_2O
[MR − (AE − BCG)] × 0.001269 = grams of replaceable hydrogen in terms of H_2O

Example. A 2.8-gram sample of a mixture of anhydrous salts (equal parts of sodium trimetaphosphate, sodium tripolyphosphate, tetrasodium pyrophosphate, and primary sodium orthophosphate) was dissolved and treated with 20 cc. of approximately 0.5 N acid. Aliquots, each representing 0.7 gram of sample, gave titration volumes, corrected to the appropriate end points, as below. All figures refer to 0.1409 N alkali.

	Cc.
BCG (to pH 4.37)	2.89
MR (from pH 4.37)	36.65
TB (to pH 8.85, less blank)	29.36
MRT (after hydration)	83.80
AE (acid equivalent)	18.90

Substituting these values:

83.80 × 0.005 = 0.4190 gram of total P_2O_5 ($o + p + m$)
(29.36 − 2.89) × 0.01 = 0.2647 gram of P_2O_5 ($o + p$)
[36.65 − (29.36 − 2.89)] × 0.01 = 0.1018 gram of P_2O_5 (o)
(18.90 − 2.89) × 0.004367 = 0.0699 gram of alkalinity as Na_2O
[36.65 − (18.90 − 2.89)] × 0.001269 = 0.0262 gram of water of constitution

By subtractions:

Meta-P_2O_5 = 0.1543 gram or 22.04 per cent
Pyro-P_2O_5 = 0.1629 gram or 23.27 per cent
Ortho-P_2O_5 = 0.1018 gram or 14.54 per cent

Composition as Oxides. The foregoing calculations give values for total phosphorus pentoxide and water of constitution. The composition in terms of oxides is completed, in the absence of any free acidity, by use of the equation

Total Na_2O in grams = grams of alkalinity + (grams of total P_2O_5 × 0.4367)

where 0.4367 is a conversion factor to obtain that untitrated sodium oxide which is bound to the (total) phosphorus pentoxide in equimolecular proportions. In the above titration example, the oxide components would then be:

36.1 per cent sodium oxide
3.7 per cent water of constitution
59.9 per cent phosphorus pentoxide

This calculation is sometimes useful in the examination of salts but is, of course, applicable only to pure sodium phosphates.

Composition as Salts. Although the method cannot identify mixtures in terms of salts of specific basicities, it provides groupings or limits of variable latitude within which the salt components must fall. Compositions in terms of salts, calculated empirically, are often convenient forms of expression in control work, but the equivocal nature of such terms should be understood. In the titration example above, an empirical composition would be:

31.7 per cent sodium metaphosphate
42.8 per cent tetrasodium pyrophosphate
0.6 per cent sodium acid pyrophosphate
24.6 per cent primary sodium orthophosphate

As is often the case, other salt compositions are possible. The presence of the polyphosphate as such is not revealed by the results, but the equivalent quantities of meta- and pyrophosphate are represented.

Modifications

The general procedure described above may be simplified if the predominating composition is known. Commercial pyrophosphates may usually be titrated directly to pH 4.2 and 9.1 without other readings, orthophosphates directly to pH 4.6 and 8.4. If metaphosphate is known to be absent, the determination of the total phosphorus content after hydration is unnecessary. Because the acid salts commonly contain no carbonate, these may often be titrated to the appropriate end points without the acidification and aeration step. Substitution of potentiometric means (*6*) for the methyl red indicator is sometimes convenient.

The general procedure is amenable to considerable rearrangement. The methyl red titration can be made, if preferred, directly on a separate aliquot or it can follow the thymol blue titration in the manner described in the earlier paper. When performed after the thymol blue titration, the sodium nitrate blank which is subtracted from the first titration should be added back to the methyl red titration because the pH at the methyl red end point is lower than that of the preceding end point. When little or no orthophosphate is present, as in commercial sodium pyrophosphate, and the methyl red titration follows the titration with oleo red B indicator, it is advisable to add a measured amount, say 1 cc., of 0.1409 N acid to the solution before adding the silver nitrate to ensure approach to the end point from the acid side. Aside from such rearrangements, the titration procedure is rather inflexible, since the pH values used as end points are based on specific conditions of phosphate concentration, salt effects, and pH-color calibration.

Table I. Schematic Outlines of Analysis

Employed by	Pyrophosphate	Orthophosphate	Metaphosphate
Britske and Dragunow (*5*)	Titrimetric measurement of acidity produced on adding excess zinc sulfate under controlled conditions	.....	.....
Kiehl and Coats (*11*)	1. Precipitated with zinc acetate under controlled conditions of acidity (pH 3.3) and concentration, then determined gravimetrically	2. Determined gravimetrically in filtrate from pyro by precipitation with magnesia mixture in presence of ammonium chloride	Known total phosphorus content permitted calculation by difference
Travers and Chu (*24*)	1. Precipitated with zinc sulfate under controlled conditions of acidity (pH 3.7–4.7) and NH_4Cl concentration, then determined gravimetrically	2. Determined gravimetrically in filtrate from pyro by neutralizing to pH 7 to precipitate zinc ammonium orthophosphate	3. Determined gravimetrically in filtrate from ortho after boiling with acid to hydrate to ortho form
Wurzschmitt and Schuhknecht (*28*)	Titrimetric measurement of acid produced on adding excess zinc ammonium iodide. Modified Britske titration	Separated by extracting phosphomolybdate complex with ethyl acetate, then determined gravimetrically	Determination of total phosphorus permits calculation by difference
Madorsky and Clark (*14*)	Determined gravimetrically by modification of Travers and Chu method (acidity at pH 2.7–2.8)	Determination of total phosphorus permits calculation by difference. Difference includes polyphosphates	Determined gravimetrically by precipitation with barium chloride at controlled acidity, pH 2.2–2.3

With obvious changes in factors, the general procedure may be used for potassium phosphates. It is not applicable to the ammonium phosphates where ammonia acts as a buffer in the alkaline range nor to the calcium phosphates where insoluble salts are formed.

Methods and Discrepancies

Aside from the acidimetric or neutralization methods, exemplified by the procedure described above, many workers have employed selective precipitations for the estimation of phosphate mixtures. Reactions of phosphate with metallic ions, particularly zinc, are frequently used. A number of these methods are briefly outlined in Table I. Limitations and difficulties of such methods are known, especially to workers closely identified with the field, but apparently have not been accorded general recognition and acceptance. An enumeration of some of these difficulties and discrepancies is believed to have use in appraising the worth of existing methods, experimental data, and concepts of composition. The enumeration is restricted to phases which may involve discrepancies of at least several per cent or the identity of phosphates. It is hoped that a wider perception of these difficulties of analysis may lead to much-needed improvements in the estimation and identification of the several phosphates.

Pyrophosphate by Direct Titration. The titrimetric method of Britske and Dragunow (*5*) has been employed in the trade for the assay of commercial pyrophosphates and is sometimes useful even in the presence of sodium silicate, carbonate, and soap. The precision of the titration is improved by using, as is now common practice, a glass or quinhydrone electrode with end point of, say, pH 3.8. Britske and Dragunow observed that the accuracy of the titration is somewhat impaired in the presence of much metaphosphate. Inaccuracy on this account, however, seems open to some doubt according to tests in this laboratory, where sodium trimetaphosphate and a sodium metaphosphate glass were employed as sources of metaphosphate. Sodium trimetaphosphate as used in this paper is that water-soluble polymer (*15*), solutions of which show no precipitate with silver or barium cations. The metaphosphate glass was prepared after Pascal (*19*) by the fusion and quick cooling of the trimetaphosphate, since this gives a product of higher metaphosphate content than the usual Graham's salt. The potentiometric titration was performed as follows:

Dissolve the sample, usually 1 gram, in 50 cc. of water, add 0.2 *N* hydrochloric or sulfuric acid in slight excess (pH less than 3.8), then dilute to 100- to 110-cc. volume. Using a glass or quinhydrone electrode titration assembly, adjust to pH 3.8 with 0.1 *N* sodium hydroxide solution. Add 40 cc. of 0.5 *M* zinc sulfate solution (equivalent by theory to 2.6 grams of tetrasodium pyrophosphate) which has been adjusted with sulfuric acid to pH 3.8. Slowly titrate the acid, liberated by the precipitation of the normal zinc pyrophosphate, with 0.1 *N* alkali while stirring vigorously until pH 3.8 is reached and maintained for at least 5 minutes. The alkali consumed is a measure of the pyrophosphate. Because the reaction as measured is not strictly stoichiometric, the standard alkali is calibrated against pure tetrasodium pyrophosphate under like test conditions.

The test results indicate that metaphosphates, at least the two varieties employed, cause no particular difficulty. In the presence of the metaphosphate glass some apparent inaccuracy is found (Table II, Test C) but this may be due to somewhat imperfect composition of the metaphosphate (Graham's salt, Test D, illustrates imperfect composition) or, as is more probable, to slight hydration during test.

In the presence of orthophosphate the Britske and Dragunow titration is subject to some inaccuracy (which increases with the orthophosphate content) because of the salt effect (*12*) of the zinc sulfate reagent upon the hydrogen-ion concentration. In addition, large amounts of orthophosphate may interfere through precipitation. The interference is accompanied by impermanent end points. The amount of orthophosphate which can be tolerated is greatly influenced by concentration and temperature. Under the above-described titration conditions, orthophosphate constituting one-half of the sample (0.25 gram of orthophosphate as P_2O_5) is tolerated but only in cool solution (Tests F to K). When orthophosphate predominates in the sample, a greater initial dilution, say to 250 instead of 100 to 110 cc., will permit completion of the titration without precipitation of orthophosphate though still subject to the salt error (Tests L to O).

Wurzschmitt and Schuhknecht (*28*) reported that the Britske and Dragunow titration was inaccurate in mixtures containing primary sodium orthophosphate, although the same inaccuracy did not occur in mixtures containing equivalent quantities of secondary or tertiary orthophosphate. The primary orthophosphate was said to result in formation of a precipitate which vitiated the pyrophosphate values; polymeric forms of orthophosphate were suggested in explanation. This exceptional phenomenon cannot, however, be confirmed in this laboratory. Under the titration conditions described above, no difference in behavior could be discerned (Tests E to O) whether primary or secondary and tertiary orthophosphate were present.

Even without resort to refinements of procedure and calibration, the Britske and Dragunow titration is seen to be useful in the analysis of mechanical mixtures of pure sodium meta-, pyro-, and orthophosphates. The method, however, is not used in the trade as a general one for sodium phosphates, because of the ability (*10*) of sodium meta- and pyrophosphate melts to form complexes or polyphosphates which are further dependent upon the heat treatment of the melts. When these complexes exist, the usual reactions of meta- and pyrophosphate with metallic ions are altered. For example, when the Britske and Dragunow method is applied to sodium tripolyphosphate, $Na_5P_3O_{10}$, titrations are obtained corre-

Table II. Pyrophosphate by Direct Titration

Test	$Na_4P_2O_7$ Taken	Added	$Na_4P_2O_7$ Found	Remarks
	Gram	*Gram*	*Gram*	
	1.0000	None	(1.000)	Calibration, 4 tests; 74.62, 74.58, 74.46, 74.68 cc. of 0.1 *N* alkali
A	0.1000	None	0.1008	.....
B	0.1000	0.9 $(NaPO_3)_3$	0.1012	.....
C	0.1000	0.9 meta glass	0.1128	.....
D	0.1000	0.9 Graham's salt	0.1546	.....
E	0.9000	0.10 NaH_2PO_4	0.9058	.....
F	0.5000	0.43 NaH_2PO_4	0.5272	Titrated at 20° C.
G	0.5000	0.50 Na_2HPO_4	0.5288	Titrated at 20° C.
H	0.5000	0.43 NaH_2PO_4	0.55+	At 32° C., unstable end point
I	0.5000	0.56 Na_3PO_4	0.54+	At 32° C., unstable end point
J	None	0.86 NaH_2PO_4	0.25+	At 32° C., unstable end point
K	None	1.00 Na_2HPO_4	0.28+	At 32° C., unstable end point
L	None	0.86 NaH_2PO_4	0.0206[a]	At 25° C., increased vol.
M	None	1.00 Na_2HPO_4	0.0202[a]	At 25° C., increased vol.
N	0.1000	0.86 NaH_2PO_4	0.1216[a]	At 25° C., increased vol.
O	0.1000	1.00 Na_2HPO_4	0.1223[a]	At 25° C., increased vol.
P	0.1000	0.50 Na_2HPO_4 + 0.50 meta glass	0.1196[a]	At 25° C., increased vol.
Q	None	1.0 $Na_5P_3O_{10}$	0.5157	First reading, unstable end point
			0.7101	Final end point; solid phase contains 0.562 gram of 0.579 gram P_2O_5 present
R	None	1.0 $Na_5P_3O_{10}$ + 20.0 NH_4Cl	0.4943	Stable end point
S	None	1.0 $Na_5P_3O_{10}$	0.4268[a]	Increased volume, no ppt. at end point

[a] Initial dilution to 250 cc. instead of 100–110 cc.

sponding to 42 to 71 per cent of pyrophosphate, depending upon test conditions. In the smaller volume (Test Q), 97 per cent of the phosphorus has been precipitated at the end point. In the larger volume (Test S), no precipitation occurs although considerable acidity is developed. Obviously the meta- and pyrophosphates have been altered by heat treatment; pyrophosphate titrations are inaccurate in the presence of polyphosphate.

The tripolyphosphate referred to in this paper is that fused and tempered phosphate obtained according to the description of Andress and Wüst (*1*). It was prepared in the laboratory from a mechanical mixture containing 72.3 per cent of tetrasodium pyrophosphate and 27.7 per cent of sodium metaphosphate, corresponding to equimolecular proportions. An aqueous solution of the powdery polyphosphate showed positive in both cases when tested by the qualitative methods described by Wurzschmitt and Schuhknecht (*28*) for hexametaphosphate and for trimetaphosphate. It coagulated albumen as does metaphosphate. However, it gave negative tests when the precipitation methods of Madorsky and Clark (*14*) for metaphosphate and for pyrophosphate radicals were applied.

The tripolyphosphate showed the following values when tested by the colorimetric pH titration method:

19.0 per cent metaphosphate as P_2O_5
38.9 per cent pyrophosphate as P_2O_5
0.0 per cent orthophosphate as P_2O_5
0.034 per cent water of constitution
42.1 per cent sodium oxide
(16.9 per cent alkalinity)

Empiric composition as salts:

72.3 per cent tetrasodium pyrophosphate
0.4 per cent disodium pyrophosphate
27.3 per cent sodium metaphosphate

The agreement of the above empiric composition with the known starting materials indicates that the corresponding tripolyphosphoric acid dissociates in substantially the same manner [3 strongly and 2 weakly dissociated hydrogen atoms (*20, 26*) but with none of the feeble dissociation shown by orthophosphate] as does an equivalent mixture of meta- and pyrophosphoric acids.

Separations with Zinc Ion. Some workers have determined the pyrophosphate radical by separating the precipitate formed with zinc ion in the presence of ammonium chloride and under conditions of controlled acidity, variously defined between pH 2.7 and 4.7. In addition, the separation is also influenced by the concentration and distribution of the several phosphoric acid radicals, the amount of zinc reagent, temperature, and time allowed for precipitation. So far as known to the authors, this multiplicity of variables has not to date been well enough defined to warrant the application of the zinc separation as a general method for sodium phosphate mixtures. This does not necessarily imply that the variables cannot be suitably defined. In this paper a few of the possible sources of inaccuracy are mentioned but no attempt is made to delineate satisfactory conditions of separation or of application.

The separation of the pyrophosphate radical seems to be particularly disturbed by the ability of sodium meta- and pyrophosphate melts to bind (*10*) many metallic ions as water-soluble complexes. A number of industrial applications of such melts are predicated upon this and related phenomena, variously described (*1, 7*) as the inhibition or retardation of numerous precipitations, the peptization of metal salts, and the stabilization of conditions of supersaturation. Although attention has been directed chiefly to reactions with calcium ion, analogous behavior may occur with other multivalent cations including zinc. This is illustrated by applications of the zinc sulfate precipitation method to known mixtures.

In these tests, the known phosphates were dissolved in 200 cc. of water, the solution was adjusted with dilute hydrochloric acid to pH 3.0 to 3.3, 20 cc. of 0.5 *M* zinc sulfate solution were added, and the acidity was readjusted with dilute sodium hydroxide solution until pH 3.3 at 25° C. was maintained for 10 minutes with final volume of 250 cc. Aliquots of the solution, clarified by filtration, were analyzed for phosphorus content by the molybdate method. The difference between the known total phosphorus and the phosphorus found in the liquid phase gives the phosphorus in the solid phase. This latter value accordingly does not involve either the composition of the precipitate or its washing technique. Tests were also made in the presence of 25 grams of ammonium chloride, since Travers and Chu recommend its addition (10 per cent) to prevent the precipitation of zinc metaphosphate.

The typical results in Table III, where the per cent recovery of pyrophosphate is given in the right-hand column, show that the presence of phosphates having metallic ion binding power may lead to gross errors when the pyrophosphate radical is sought by the reaction with zinc cation. In view of the many variables, the test conditions used above give a very limited portrayal of the possibilities of the reaction but they seem sufficient to indicate that the determination of pyrophosphate in this manner warrants more than casual investigation and description.

In some limited applications, of course, the method employing the zinc pyrophosphate precipitation is appropriate. A specific instance is seemingly afforded in the work reported by Kiehl and Coats (*11*) in their study of the hydration of sodium metaphosphate in alkaline solution. From the mode of preparation and the reported water of crystallization, the metaphosphate under study appears to have been the trimetaphosphate, although erroneously designated as the monometaphosphate as noted by Nylen (*18*). The trimetaphosphate has no metallic ion binding power (*26*) nor does it form a precipitate with zinc ion (*15*) under the test conditions as may the glassy metaphosphate. From the manner of preparation, the presence of polyphosphates or complexes with their attendant difficulties may be excluded. Moreover, the difficult condition (*28*) of small amounts of pyrophosphate was not encountered because the end products of the hydration were equimolecular quantities of pyro- and orthophosphate. Although the separation as employed appears to be suitable for the particular problem at hand, its application as a general method was not investigated nor implied and would be manifestly inappropriate.

On the other hand, Travers and Chu (*24*) reported commercial glacial metaphosphoric acid to be a mixture of the meta and ortho forms and found 18.6 per cent of sodium oxide. The ortho form was deduced from acidimetric titrations

Table III. Separations with Zinc Ion

Taken				Found	
$Na_4P_2O_7$	Other phosphate	Total P_2O_5	NH_4Cl	Solid phase	P_2O_5 in solids / P_2O_5 in pyro
Mg. P_2O_5	*Mg.* P_2O_5	*Mg.*	*Grams*	*Mg.* P_2O_5	%
106.8	None	106.8	None	104.7	98.0
106.8	None	106.8	25	91.4	85.6
53.4	None	53.4	None	51.8	97.0
None	347.0 Graham's salt	347.0	None	None	...
None	347.0 Graham's salt	347.0	25	None	...
53.4	347.0 Graham's salt	400.4	None	None	0.0
106.8	347.0 Graham's salt	453.8	None	96.3	90.2
106.8	347.0 Graham's salt	453.8	25	None	0.0
106.8	138.4 Graham's salt	245.2	None	127.7	119.5
106.8	138.4 Graham's salt	245.2	25	None	0.0
None	288.5 $Na_5P_3O_{10}$	288.5	None	None	...
None	288.5 $Na_5P_3O_{10}$	288.5	25	None	...
53.4	288.5 $Na_5P_3O_{10}$	341.9	None	None	0.0
106.8	288.5 $Na_5P_3O_{10}$	395.2	None	111.2	104.1
106.8	288.5 $Na_5P_3O_{10}$	395.2	25	None	0.0
106.8	115.4 $Na_5P_3O_{10}$	222.2	None	119.5	111.9
106.8	115.4 $Na_5P_3O_{10}$	222.2	25	None	0.0
53.4	347.0 $(NaPO_3)_3$	400.4	None	53.0	99.2
53.4	312.6 Na_2HPO_4	366.0	None	52.8	98.9

using methyl orange and phenolphthalein indicators which showed the presence of a multibasic acid; pyrophosphoric acid was eliminated because of the absence of precipitate with zinc ion, thus leading to the assumption that the acid was the orthophosphoric form. As shown in Table III, however, the absence of a precipitate may not be conclusive evidence of the absence of pyrophosphate. Moreover, the possibility of polyphosphates or complexes was not eliminated.

When tested in this laboratory by pH titrations, a fresh sample of a commercial C. P. grade of metaphosphoric acid (glassy sticks with little white opaque coating) showed

25.5 per cent metaphosphate as P_2O_5
36.8 per cent pyrophosphate as P_2O_5
9.2 per cent orthophosphate as P_2O_5
17.2 per cent base as sodium oxide
11.1 per cent water of constitution

The free acid content was equivalent to 32.1 per cent phosphorus pentoxide. In a supplementary test the orthophosphate content was found to be 9.6 per cent by a modification of the molybdate precipitation method of Hitchens and McCauley (*8*). That the glacial acid is more than the mixture of meta and ortho forms, which Travers and Chu report, is further indicated by its behavior in the Britske and Dragunow titration where the alkali consumed corresponds to 28.7 per cent pyrophosphate. Although the titration is not accurate for this type of material, the large alkali consumption is a proof that more than meta- and orthophosphoric acids are present.

In view of the interference introduced by the presence of polyphosphates and polymers, the question naturally occurs as to the frequency with which this may be encountered. Because metaphosphates are seldom, indeed rarely, free from complexes having zinc ion binding power, the presence of metaphosphate (or of polyphosphate) in any phosphate mixture may be regarded as a potential source of more or less difficulty in the determination of pyrophosphate using zinc ion.

Metaphosphate. The direct gravimetric determination of metaphosphate using barium chloride as precipitant has been employed by Holt and Myers (*9*) and by Madorsky and Clark (*14*). The test, however, is not a generic one because of differences in behavior of the polymeric forms. Thus, the determination surely would not include trimetaphosphate, which forms no precipitate with barium ion (*15*) under the test conditions. The full significance and manner of application of the barium precipitation seem incompletely developed at this time.

Holt and Myers reported polymeric forms of pyrophosphoric acid, (1) a simple form obtained by displacement from lead pyrophosphate and (2) a polymer of formula between $(H_4P_2O_7)_4$ and $(H_4P_2O_7)_5$ which was obtained by dehydration of orthophosphoric acid. The latter form, however, is a mixture of acids in a condition of equilibrium as had been adduced by Berthelot and André (*4*) and recently described by Lum, Malowan, and Durgin (*13*). An equilibrium constant has been determined (*6*) by the use of pH titrations.

For the examination of the metaphosphate reactions, Graham's salt is frequently used as a source of the sodium salt. When prepared by the usual fusion of primary sodium orthophosphate at red heat, the conversion to metaphosphate is more or less incomplete because of the difficulty with which the last bit of water is expelled. As a result, solutions of the melt are somewhat acid in reaction. The residual water of constitution can be readily estimated by quickly dissolving a portion of the chilled melt in cold water, adding an excess of silver nitrate reagent, and titrating with standard sodium hydroxide solution using methyl red indicator.

From their examination of Graham's salt, Treadwell and Leutwyler (*25*) concluded that the metaphosphate is always attended by an appreciable amount of orthophosphate (10 per cent NaH_2PO_4 in an example) which is present as a reaction product; a theoretical explanation involving splitting of molecules was given. The orthophosphate was said to be best determined from its buffer action by measuring the caustic consumption from pH 4.3 to 9.5. Although this titration is an excellent tool in the examination of sodium metaphosphate for composition, it is not conclusive as a measure of orthophosphate unless the absence of pyro- and polyphosphates or complexes has been fully assured, for which purpose the electrometric titration curves as used seem inadequate.

When Graham's salt was prepared in this laboratory by heating primary sodium phosphate (25 grams) in an oven at 700° C. for an hour, an analysis of the chilled melt by the pH titration method showed the presence of 3.8 per cent pyrophosphate as P_2O_5 but no orthophosphate, while the water of constitution was 0.46 per cent. Moreover (Table II, Test D), Graham's salt (from the above preparation) behaves in the Britske and Dragunow titration as though it contained some pyro- or polyphosphate; such behavior would not be expected if the buffering salt were orthophosphate. From this and many similar observations, the conclusion is reached that, contrary to Treadwell and Leutwyler, orthophosphate is not a reaction product of the dehydration of primary sodium phosphate at red heat, even though important amounts of buffer salt are present.

Salih (*21*) reported that a hexametaphosphoric acid, obtained by displacement from the lead salt, showed 4 strongly and 2 weakly dissociated hydrogen atoms. Such dissociation, if true, would invalidate the colorimetric pH titration method. However, Treadwell and Leutwyler contest (*25*) Salih's work and find metaphosphoric acids to be strongly dissociated (*26*), an observation in agreement with that of the authors and of Rudy and Schloesser (*20*). The value (pH 9.1) given by Salih for the hydrogen-ion concentration of a 0.1 *N* solution of the sodium salt suggests that the acid under examination may have included phosphoric acid of a higher degree of hydration than the meta stage.

Wurzschmitt and Schuhknecht (*28*) recently described qualitative tests for the detection of ortho-, pyro-, hexameta-, and trimetaphosphate. Although the tests are said to be applicable to phosphates in the presence of one another, this phase seems insufficiently developed and no known mixtures were included in the tabulated results showing test performance. Unfortunately, many reactions, even though suitable for the identification or estimation of phosphates occurring singly, are inadequate when two or more phosphates are present.

An example of such inadequacy is afforded by the qualitative test where the presence of phosphate in the filtrate from a precipitation with barium chloride in a slightly alkalinized solution of the sample is said to indicate the presence of trimetaphosphate. In parallel tests where sodium orthophosphate, Graham's salt, and a 1 to 5 mixture of the two were treated as directed by Wurzschmitt and Schuhknecht, the filtrate corresponding to the 1 to 5 mixture showed a prompt and strong test for phosphate. On standing, all three of the treated filtrates showed the yellow phosphomolybdate precipitate; the least precipitate corresponded to the orthophosphate sample while by far the most precipitate corresponded to the 1 to 5 mixture. Although the strong test shown by the 1 to 5 mixture would indicate, according to directions, the presence of much trimetaphosphate, such a conclusion obviously is not justified in this case. Instead, the prompt positive test seems only to indicate that the precipitation of orthophosphate with barium ion may be retarded when Graham's salt is present, thus allowing orthophosphate

to appear in the filtrate. Analogous interference in the precipitation of pyrophosphate radical with zinc ion has been noted in Table III.

In addition to being thus exposed to false positive or false negative results in the presence of phosphates having metallic ion binding power, the qualitative tests of Wurzschmitt and Schuhknecht are confined to the four phosphate radicals with no cognizance being taken of complexes or polyphosphates. Because of these limitations, employment of the tests in a general way leaves much to be desired. Thus commercial sodium metaphosphates were identified (*28*, Table I) only as the hexametaphosphate with occasional traces of orthophosphate although very considerable amounts of buffering salt have always been found when such phosphates have been tested acidimetrically.

A domestic commercial purified sodium metaphosphate reagent, which the manufacturer affirms to be typical, was recently found by pH titrations to have an empiric salt composition of 11.9 per cent sodium metaphosphate, 86.8 per cent sodium acid pyrophosphate, and 1.1 per cent tetrasodium pyrophosphate. The metaphosphate content of 11.9 per cent was essentially the water-insoluble variety.

Nomenclature. Determinations of the phosphoric acids by various modes of attack introduce an ambiguity in nomenclature. In the acidimetric method, meta-, pyro-, and orthophosphoric acids are regarded as phosphorus pentoxide in three stages of hydration or union with water (1, 2, and 3 moles of water per mole of phosphorus pentoxide) which can be estimated by means of a 3-step neutralization. The test result shows phosphorus pentoxide hydrated to some particular degree which is expressed in terms of the three stages.

On the other hand, if the determination is done by the separation and estimation of the acid radicals—for example, P_2O_7 using zinc ion or $(PO_3)_n$ using barium ion—the phosphoric acids cannot be considered as a ternary group except perhaps as they may be regarded as ortho-, poly-, and metaphosphoric acids, in which case pyrophosphoric acid is deemed (*16*) to be a member (bipolyphosphoric acid) of the series of polyphosphoric acids. In any event, consideration cannot be confined to acid radicals of the ortho-, pyro-, and metaphosphates but must be extended (*13*, *26*) to include the more complex combinations. Failure to recognize this situation has been common. As a result much work that has appeared regarding composition and transformations of the phosphates is of little value unless the methods of analysis are first examined. Until the number and identity of the acid radicals of the polyphosphates—and also of the polymers of metaphosphoric acid—are better defined, the practice of determining any phosphate radical by difference is rarely admissible.

Because of these differences in the manner of attack, the value for pyrophosphate, as an example, when determined by the acidimetric method may at times involve complexes or polyphosphate and thus be higher than the pyrophosphate found by precipitations which would reflect only the pyrophosphate radical, P_2O_7. Such ambiguity, involving definition of terms, has been a not infrequent source of discrepancies in phosphate data since workers have variously employed acidimetry, precipitations, and at times, jumbled combinations of the two.

Some workers have employed x-ray diffraction methods in the examination of sodium phosphates for the purpose of identifying the chemical compounds. Thus Andress and Wüst reported (*1*) differentiation of the more distinctly crystalline compounds but found (*2*) the x-ray method unsuitable for the examination of certain vitreous phosphate complexes which are essentially amorphous.

Although the pH titration method cannot perform the function of distinguishing complexes or polyphosphates from an equivalent mixture of meta- and pyrophosphates, it has been found useful in practice when applied to a wide variety of phosphates encountered in manufacture and research. In these applications the method serves to follow molecular dehydrations in heat treatments or molecular hydrations in contact with water, to determine the base-acid ratio or the composition in terms of the oxides, and to provide limits within which the salt composition must fall.

The acidimetric method appears to be applicable to the assay of phosphoric acids and their sodium salts excepting only those compounds, usually high in the meta form, which hydrate under the test conditions. Such hydration errors are small or negligible except in the case of those acids, practically acid anhydrides, containing 88 to 90 per cent of phosphorus pentoxide and higher, which are not brought into aqueous solution without considerable hydration. The error in this case has been readily discerned because the sum of the phosphorus pentoxide and the combined water, calculated from the titrations, then exceeds the weight of sample.

Summary

Acidimetric titrations, previously used for strong phosphoric acids, are applied to the analysis of sodium phosphates where they provide a useful classification in terms of meta-, pyro-, and orthophosphate. Polyphosphates, if present, titrate as mixtures of meta- and pyrophosphate. Sources of discrepancies, when using various methods of phosphate analysis, are enumerated. In those methods where the estimation of phosphate radicals is accomplished by precipitations using metallic ions, consideration cannot be confined, as has been common, to acid radicals of the meta-, pyro-, and orthophosphates but must be extended to embrace the phosphate complexes or polyphosphates. In the presence of polyphosphate, analysis by acidimetry and by precipitation introduces a potential source of confusion in terminology. The determination of phosphate radicals, using metal salts as precipitants, may be subject to interference because of the metallic ion binding power of sodium meta- and pyrophosphate melts. Thus the titrimetric or gravimetric estimation of the pyrophosphate radical using zinc ion is exposed to inaccuracy in the presence of polyphosphate. Other causes of substantial discrepancies in phosphate identity or in analytical data are considered.

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Determination of Succinic Acid in Plant Tissues

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Succinic acid is extracted from plant tissue with ether, freed from contamination with other substances by oxidation, converted into its anhydride, and condensed in toluene solution with *p*-toluidine to the insoluble crystalline succinyl-*p*-toluide. The properties of this substance are such as to permit of substantially quantitative isolation, and of identification by means of the melting point and crystalline form. An empirical solubility correction and a conversion factor are provided that lead to average recoveries of the order of 99 per cent, and single determinations can be made within ±5 per cent over the range from 1 to 20 mg. of succinic acid. The only known interfering substance is α-ketoglutaric acid.

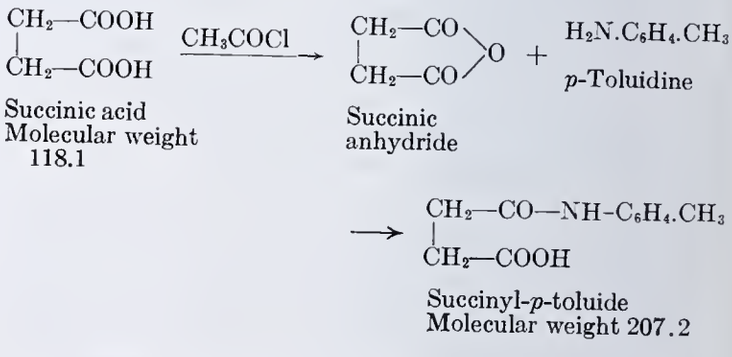
SUCCINIC acid is now known to be a metabolite involved in the respiration system of certain animal tissues (*1, 2, 8, 14, 15*), and current speculation suggests (*4, 21*) that it may play an analogous part in plants as well. Accordingly, the recognition and, if possible, the accurate determination of succinic acid in plants become a matter of concern to the further development of our understanding of the physiological functions of the organic acids that form so important a part of most plant tissues.

Early methods of determining succinic acid, most of which depend on the precipitation of an insoluble inorganic salt, were thoroughly reviewed in 1909 by von der Heide and Steiner (*11*) who recommended the use of the silver salt as being the most satisfactory. More recent modifications of the silver salt method, designed for application to the analysis of animal tissues, have been described by several investigators (*5, 7, 9, 16*). During the past few years, however, attention has been directed chiefly to methods that depend on the oxidation of succinic acid in the presence of an enzyme that occurs in muscle tissue (*2, 12, 22*). These methods are especially suited to the estimation of the minute amounts of succinic acid that are encountered in the small samples of animal tissue employed for the study of the details of metabolism.

For work with plant tissues, none of these methods is completely satisfactory. Our knowledge of the qualitative composition of the mixture of organic acids present in most plants is very limited and salt precipitation methods are specific only under the most carefully controlled conditions. The enzyme method, although probably specific, requires the use of the Warburg manometric apparatus and of a highly specialized technique, and its applicability to the conditions encountered in the plant field is still to be established. It seemed desirable, therefore, to develop a simple method which would permit the isolation of a characteristic derivative of succinic acid in order to provide both qualitative and quantitative evidence of the tissue composition.

Auwers, in 1896 (*3*), observed that anhydrides of organic acids condense with certain aromatic amines to give insoluble crystalline derivatives. Succinic acid is readily converted into its anhydride when heated with acetyl chloride (*6*), and the condensation product of succinic anhydride with *p*-toluidine mentioned by Auwers is a well-crystallized substance that is almost insoluble in toluene and possesses excellent properties for quantitative isolation and for identification.

The reactions involved are:



The theoretical yield from 1 mg. of succinic acid is 1.75 mg. of toluide. Although the use of amines of even larger molecular weight would appear to be desirable, none of those tested gave condensation products with as favorable properties as the *p*-toluide.

Preparation of Organic Acid Fraction

Fresh plant tissue is prepared for analysis by being dried in a ventilated oven at 80° C. and is then ground to a powder. Of this, 1.0 gram is accurately weighed and is extracted with ether as described by Pucher, Wakeman, and Vickery (*17*). The ether extract is treated with 25 ml. of water and the ether is evaporated. Troublesome frothing is sometimes encountered if alkali is added before evaporation. The solution is diluted with 25 ml. of water,

2 ml. of 5 *N* sodium hydroxide are added, and the flask is agitated until all residue is dissolved. The solution is acidified by the addition of 3.5 ml. of 18 *N* sulfuric acid; 5 ml. of cold saturated barium hydroxide solution (to provide a solid phase to assist in the removal of undesirable contaminants) and 0.5 gram of asbestos are added and the mixture is boiled gently for a few minutes, cooled, and filtered with suction through a layer of asbestos into a 150-ml. beaker marked at a volume of 75 ml. (A convenient apparatus for this and subsequent filtrations is constructed from a small bell jar with side tubulature for the vacuum line and top tubulature for the introduction of the funnel that supports the Gooch crucible. The bell jar rests on a lubricated glass plate on which the beaker is placed on a small block of wood with a hole to accommodate it. A small desiccator can be substituted for the bell jar.)

The flask and asbestos are washed repeatedly with small amounts of water until a total volume of 75 ml. of filtrate is reached. The solution is then heated for 10 minutes in a boiling water bath, 30 to 35 ml. of 1.5 *N* potassium permanganate are added, and heating is continued for about half an hour. From time to time the solution is stirred and examined (spot test on filter paper) for the presence of excess permanganate, more being added if necessary. An excess must still be present at the end of the oxidation. The hot solution is then treated with a freshly prepared 20 per cent solution of anhydrous sodium sulfite until decolorized and 0.5 ml. excess is added.

After being evaporated to about 25 ml. on the steam bath, it is filtered into the tube of a continuous liquid extraction apparatus and is extracted with alcohol-free ether overnight. A suitable apparatus, modified from that of Handorf (*10*), which is placed in an ordinary Soxhlet apparatus has been described by Vickery and Pucher (*20*), and a somewhat similar device is described by Quick (*18*). The ether extract is treated with 5 ml. of water and most of the ether is evaporated in a water bath, the aqueous residue being finally boiled for a few minutes. It is then chilled and washed into an Erlenmeyer flask fitted with a standard taper joint for the subsequent attachment of a reflux condenser, and is evaporated, finally on a steam bath, to dryness. The evaporation of the succinic acid solution must be conducted with great care to avoid loss by volatilization. A hot plate may be used in the early stages, but not at the end.

In order to ensure the complete oxidation of all organic substances except succinic acid, the dry residue is treated with 0.2 ml. of concentrated hydrochloric acid and 0.1 ml. of concentrated nitric acid and is again evaporated to dryness on the steam bath. (Tests with 2.5 times these quantities of nitric and hydrochloric acid showed that no detectable loss of succinic acid occurs.) The flask is then cooled and is placed in a vacuum desiccator charged with sulfuric acid and provided with a small container of solid sodium hydroxide, and is left under vacuum until all traces of volatile acid have been removed; this usually requires 4 to 5 hours, but it is best to allow the sample to remain overnight.

Dehydration of Succinic Acid

The dry residue of succinic acid is treated with about 0.5 ml. of redistilled acetyl chloride (boiling point, 50° to 54° C.) and is heated for an hour with occasional shaking under reflux in an all-glass apparatus in an oil bath at 55° to 60° C. Protection from moisture is desirable. The flask is then raised out of the oil bath and allowed to cool for 3 or 4 minutes and is placed in a desiccator that contains sulfuric acid and a container of solid sodium hydroxide, and allowed to stand overnight at room temperature. The desiccator must not be evacuated. Losses of succinic anhydride have invariably been encountered if attempts are made to hasten the rate of evaporation of the acetyl chloride by the use of heat or vacuum and this step has been found to be one of the most critical for the success of the analysis.

Formation of Succinyl-*p*-Toluide

Five milliliters of boiling toluene are added to the flask, which is then covered with a glass bulb or watch glass, and is heated to maintain boiling for about 5 minutes; 50 mg. of tricalcium phosphate are added as filter-aid and the solution is filtered with gentle suction into a 10-ml. beaker through a 1.5 ml. dry Gooch crucible provided with a thin mat of asbestos. The flask is washed twice with 1 ml. of boiling toluene and the crucible is finally rinsed with 0.5 ml. of the same solvent. The clear filtrate is treated with 0.5 ml. of a 6 per cent solution of *p*-toluidine in toluene (reagent preserved in the refrigerator) and the mixture is stirred with a fine rod. This is removed, rinsed with a few drops of toluene, and the covered beaker is allowed to stand at room temperature for 5 minutes and is then warmed in an oil bath at 60° to 65° C. for 10 minutes. The beaker is then removed from the bath, allowed to cool to room temperature for 10 minutes, chilled in a bath of water at 4° to 5° C., and placed in the refrigerator for an hour. After about 15 minutes, a rod is introduced and the crop of crystals is occasionally stirred.

Filtration is accomplished on a 1.5-ml. dry, weighed Gooch crucible furnished with a thin asbestos pad. The filtrate is collected in a graduated cylinder to provide a check on the volume and is used as necessary, together with a rubber policeman, to complete the transfer of the crystals to the filter. It is kept cold during the filtration and other manipulations by being immersed in a beaker of ice water. The precipitate is finally sucked dry, washed twice with 1 ml. of a saturated solution of succinyl-*p*-toluide in toluene, dried at 105° C. for half an hour, and weighed.

The wash fluid employed is prepared from a sample of pure succinyl-*p*-toluide made by treating 0.5 gram of recrystallized succinic anhydride (*19*) with 0.6 gram of *p*-toluidine in 15 ml. of chloroform. The mixture is warmed to the boiling point for 30 minutes and is then cooled to 5° C. The crystals are filtered and washed with cold chloroform and should melt at 178° C. uncorrected. Of this material, 50 mg. are heated with 500 ml. of toluene for a short time and then chilled in the refrigerator overnight; after being filtered the solution is kept at low temperature.

Calculation of Results

Succinyl-*p*-toluide is not entirely insoluble in toluene under the conditions employed nor is the conversion of succinic acid into this product quantitative. However, the conditions have been adjusted so as to admit of repetition in exact detail and an empirical solubility correction and a factor to convert the results into substantially accurate determinations of succinic acid have been established. The weight in milligrams of the succinyl-*p*-toluide derived from 1 gram of tissue, when inserted into the following formula, yields the percentage of succinic acid.

(Weight of succinic-*p*-toluide + 0.34) 0.059 = per cent succinic acid. The data upon which this formula rests are discussed below.

Identification of Succinic-*p*-Toluide

Although when prepared from pure succinic acid, this product is colorless and well crystallized, samples obtained from plant tissues are occasionally dark in color and may not be entirely typical in appearance. Proof of identity is therefore desirable, as well as some index of purity. The melting point has been found to provide this information and, together with the appearance under the microscope, serves for complete identification. The quantity of material available is frequently so small, however, that special technique is required for the preparation of crystals of typical appearance.

A convenient method is to treat the dry product in the crucible with 1 ml. of acetone, which dissolves it readily, and draw the solution gently through into a 5-ml. beaker placed beneath it in the filtration apparatus. The asbestos is stirred with a fine rod and transfer is completed with a second equal quantity of acetone. Suction is maintained until the whole of the acetone has evaporated, and the beaker and crucible are then dried at 105° C. for a few minutes to remove the last traces of acetone. One milliliter of methylamyl acetate (methylisobutylcarbinol acetate) is added to the beaker, which is then warmed on a hot plate until the residue is dissolved (1 ml. of boiling methylamyl acetate will dissolve about 10 mg. of succinyl-*p*-toluide), 1 ml. of boiling toluene is added, and the mixture is filtered through the original crucible into a 10-ml. beaker. The original beaker is rinsed 3 times with 2-ml. portions of hot toluene which are also drawn through the filter. The filtrate is heated in case crystallization has begun, and is then chilled in the refrigerator and, when cool, is seeded with a tiny crystal of pure succinyl-*p*-toluide and stirred until crystallization begins. After being allowed to stand overnight, the crystals are filtered on a small Hirsch funnel on hardened filter paper, washed with cold toluene, and dried at 105° C. A sample of the product is mounted in water for examination under the microscope and the melting point is determined.

A product that melts completely to oil at or above 173° C. uncorrected, as measured with a long-stem thermometer, is

Table I. Yield of Succinyl-*p*-Toluide from Succinic Acid

Succinic Acid Taken	Toluide Found	Mean Ratio of Toluide to Acid (Theory 1.75)
Mg.	*Mg.*	
1.00	1.33	
	1.29	
	1.31	
	1.34	1.32
1.25	1.73	
	1.72	
	1.77	
	1.77	1.40
5.00	8.21	
	8.04	
	8.34	
	8.25	1.64
20.00	33.94	
	33.73	
	34.17	
	33.90	
	33.55	1.70

regarded as being satisfactorily pure. In general the melting point lies between 175° and 177° C.; the pure substance melts at 177° to 178° C.

The crystals are long narrow parallel-sided prisms or needles, the ends of which are usually square. Occasionally somewhat stout rectangular, but often broken, prisms are formed. Comparison with authentic pure material crystallized from the same solvent must be made until familiarity with the appearance of the substance is acquired.

When only about 2 mg. of the product are available, recrystallization may be effected with 5 ml. of boiling toluene alone and the filtration omitted. Under these circumstances typical crystals are less easily secured.

Table II. Recovery of Succinic Acid as Succinyl-*p*-Toluide

Succinic Acid Taken	Toluide Found	Toluide Calculated	Succinic Acid Recovered: Uncorrected	Corrected for solubility	Corrected for solubility and by factor
Mg.	*Mg.*	*Mg.*	%	%	%
1.00	1.34	1.75	76.4	95.8	99.1
1.25	1.75	2.19	79.8	95.3	98.7
2.50	3.80	4.39	86.7	94.4	97.7
5.00	8.21	8.77	93.6	97.5	100.9
10.0	16.95	17.54	96.6	98.5	102.0
20.0	33.88	35.08	96.6	97.6	100.9

Recovery of Pure Succinic Acid

Table I shows the results of analyses in which from 1.0 to 20.0 mg. of succinic acid were taken. Aliquot parts of a standard solution were evaporated to dryness in the flasks and were then treated as described. The replications show that consistent results are readily secured, but the recoveries of the smaller amounts of acid are low. Clearly the solubility of the toluide, under the conditions of the analysis, cannot be neglected. Solubility determinations, made by evaporating the saturated solution of the toluide in toluene and by calculation from recoveries of toluide derived from weighed amounts of succinic anhydride, led to a mean value of 0.34 mg. in 7 ml. of toluene at the working temperature of 5° to 9° C. This figure is empirical, since the values calculated from recovery experiments contain the errors due to losses in the manipulations. It of course applies only to analyses conducted exactly as described. The observed solubility in 7 ml. of toluene was close to 0.30 mg.

Justification for the use of this correction is shown in Table II which gives the results of a series of determinations, each figure being the mean of four or more separate values. Addition of the solubility correction brings all the results obtained over the range 1 to 20 mg. of succinic acid to essentially the same level of recovery. The maximum discrepancy even for the smallest quantities is only 5 per cent. The mean of all is 96.5 ± 1.6 per cent. To allow for losses over the entire procedure, including failure to accomplish complete conversion of the acid to its anhydride and to bring about complete condensation to the toluide, an empirical factor is used to convert the weight of the toluide to succinic acid. The theoretical value of this factor is 0.570; if this is increased to 0.590—that is, by 3.5 per cent—the recoveries shown in the last column of the table are secured. The mean of these is 99.9 ± 1.6 per cent over the entire range of quantities considered.

Succinic Acid in Plant Tissues

A series of determinations of succinic acid in several tissues is shown in Table III. This acid is a minor constituent as compared with the malic or citric acid such tissues as these usually contain, and its significance as a metabolite still awaits full explanation. From the point of view of the present paper, it is necessary only to establish the validity of the results as determinations of succinic acid. Aside from the reproducibility, which is satisfactory, the most useful criteria are the melting point, the crystalline form, and the color of the product. The melting point of material derived from pure succinic acid is 177° to 178° C. uncorrected. However, when working with small quantities under the conditions described, melting points as low as 173° C. are frequently encountered, and the preparations are not always white after being dried. Moreover, the melting points of the products in the replicate tissue analyses in Table III vary within these limits and there are differences in the color, although there is no serious variation in the yields.

Table III. Determinations of Succinic Acid in Leaf Tissues

	Wt. of Sample	Wt. of Toluide	Succinic Acid	Melting Point (Uncorr.)	Color of Toluide
	Grams	*Mg.*	%	° *C.*	
Tobacco	1.0	9.03	0.55	173	White
	1.0	9.24	0.56	177	Pale brown
	0.5	4.09	0.52	175	Brown
	0.5	4.21	0.53	173	White
Bryophyllum	0.5	1.39	0.19	173	Brown
	1.0	2.91	0.20	176	White
Maize	1.0	3.27	0.21	174	White
	1.0	2.74	0.18	173	Pale brown

Table IV. Recovery of Added Succinic Acid

	Succinic Acid Present	Succinic Acid Added	Recovery
	%	*Mg.*	%
50 mg. of citric + 100 mg. of malic acid	0.0	10.0	99.6
	0.0	10.0	103.8
Tobacco leaf	0.18	20.0	100.3
	0.57	5.0	102.9
	0.57	5.0	102.9
	0.50	2.5	94.0
	0.50	2.5	104.0
Rhubarb leaf	0.03	5.0	99.8
	0.03	5.0	104.0

That a contamination of the toluide that depresses the melting point to 173° C. is without significance as regards the validity of the analytical data is apparent from the results of a test in which succinic acid was added to a sample of rhubarb leaf blade tissue which yielded only 0.17 mg. of apparent toluide per gram; the succinic acid content was thus of the order 0.03 per cent or less. Five milligrams of succinic acid were added to 1 gram of this and recovered. The resulting toluide weighed 8.30 mg., the equivalent of 5.1 mg. of succinic acid, and melted at 173° C. If it be assumed that the whole of the product derived from the tissue itself represents a contamination, the recovered toluide would have contained 2.5 per cent of impurity, a quantity that would not significantly affect the accuracy of an analysis by the present method, and its melting point fell within the limits found when dealing with recoveries of succinic acid taken as such rather than added to tissue. Additional individual data for the recovery of succinic acid from a mixture with other acids and from tissues of different succinic acid content are shown in Table IV; all save one are within ±5 per cent.

Discussion

Comment on the steps of the procedure is perhaps superfluous, save to say that the technique of working with small amounts of material must be acquired by patient practice. The conditions under which the toluide is crystallized have been described in great detail, since the appearance of the final product is a matter of concern in the identification, and the validity of the calculation of the results rests upon adherence to the directions. Success is dependent on the complete removal of acid and of acetyl chloride before the condensation is attempted; if the product separates at once when the toluidine is added, this has not been accomplished and the analytical results are worthless. Precipitation at this point should not begin for several minutes, depending on the amount of succinic anhydride present. However, no tendency towards supersaturation has been noted when the solution in toluene is chilled; the product at this stage may be crystallized or may separate in a granular form. The certain production of typical crystals requires the recrystallization step.

Succinyl-*p*-toluide is too soluble in ether, chloroform, or other common solvents to permit of convenient recrystallization of small amounts. It dissolves in hot methylamyl acetate easily but separates sluggishly when the solution is cooled; accordingly the filtration is readily accomplished and, by means of the addition of toluene, a fairly complete final separation is brought about after the solution is seeded and chilled. The loss is small and sufficient material for a melting point can be obtained from as little as 1 mg. under the conditions described. It is important to note that these conditions are selected to bring about only a minimum purification, so that the melting point of the recrystallized product may as truly as possible indicate the purity of the material weighed.

The specificity of the method depends upon the success with which the toluene solution has been freed from substances, other than succinic anhydride, that condense with toluidine. The original extraction of the organic acids from the tissue provides for the separation from carbohydrates and amino acids—e. g., glutamic acid— that might yield succinic acid on oxidation. Other organic acids are destroyed by the oxidation with permanganate and the remaining traces of interfering substances in the second ether extract are destroyed by aqua regia. Omission of this step frequently leads to the formation of an oily condensation product.

The most critical points of the procedure are the evaporations, particularly that of the excess of acetyl chloride. For this reason redistilled pure reagent must be employed and access of moisture should be prevented but, owing to the volatility of the anhydride, the use of elevated temperature or vacuum is inadmissible.

The preparation of the plant tissue for the analysis requires no especial remark save that, in the case of material such as seed tissue that contains true fats, previous extraction with ether to remove the fat should not be attempted because of the danger of loss of succinic acid. Fat itself does not interfere, since it is removed during the preparation of the solution for oxidation. A test of tobacco-seed oil subjected to the whole procedure gave a hardly weighable trace of condensation product. However, if removal of fat previous to the succinic acid determination is necessary, the extraction should be done with petroleum ether. No loss of succinic acid is to be anticipated if this solvent is used.

The only substance present in plant tissues that would be expected to interfere with the present method is α-ketoglutaric acid. This substance is quantitatively oxidized to succinic acid by permanganate (*13*) and its properties are such that it would follow the succinic acid to the oxidation step. Accordingly the results of the present method as applied to plant tissues include α-ketoglutaric acid. However, only traces of this substance have been reported in plants in the few cases where it has been identified at all, and in any case, according to present theory, it is closely related in metabolism to succinic acid. Attempts to provide at least qualitative tests from which some estimate of the significance of the influence of this possible contaminant may be secured are now in progress.

The method is designed to deal with samples of tissue not larger than 1 gram; practical difficulties are encountered if larger samples are taken. The lower limit of certain identification of succinic acid is about 1 mg. and the quantities of reagents are adjusted to care for a maximum of 25 mg. The range of the method is thus from about 0.1 to 2 per cent of succinic acid in the dry tissue; for tissues richer than this smaller samples should be taken. Between these limits a precision of ±5 per cent for a single determination should be readily attainable.

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Use of Silicomolybdic Acid Indicator before Volumetric Oxidation of Iron

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IN THE most generally used volumetric determinations of iron an excess of stannous chloride must be destroyed with mercuric chloride before the standard oxidizing agent reacts with the ferrous ion. However, unless care is exercised in limiting the amount of the reducing agent the appearance of a gray colloidal mercury precipitate will serve as a signal for discarding the sample. In the electrometric determination an inflection point denotes the end of oxidation of the stannous chloride and the beginning of oxidation of the ferrous ion, while a second inflection point at the end of the titration of the ferrous ion takes the place of a color change in the usual titration. Up to the present no method has employed an inside color indicator where the first inflection point should appear, although many indicators of the diphenylamine and other types have been used at the second inflection point.

In the present method silicomolybdic acid indicator, which is reduced to molybdenum blue by stannous chloride, replaces the troublesome mercuric chloride, since the blue color disappears just before the oxidation of ferrous ion begins. The volume of standard dichromate used between this "blue end point" and the appearance of the red of *N*-phenylanthranilic acid indicator is equivalent to the iron being determined.

Although molybdenum blue formed under the existing conditions disappears just after the oxidation of the excess of stannous chloride and thus before oxidation of the ferrous ion begins, it was found by Wu (*2*) that with a mixed phosphomolybdic-phosphotungstic acid indicator the disappearance of molybdenum blue could be used to detect the end of oxidation of ferrous ion with either hydrogen peroxide or weak dichromate solutions. Were this the case under the conditions that obtain here, the method of the authors would not have been possible.

Careful titrations of solutions made from pure electrolytic iron with those made from pure potassium dichromate have shown that with the new method equivalency is maintained within one part in a thousand.

Reagents and Apparatus

In all work check runs were made with separately distilled water and are reported separately. Appropriate blank runs were made with about 0.50 ml. of the iron solutions to furnish data for calculation of the actual blanks, which were found to be about 0.06 ml. in terms of 0.1 *N* dichromate. The blank runs, using slightly divergent volumes from 0.50 ml. of iron, were corrected by simple proportion to fit exactly that volume of iron. In the same way in the regular runs the volume of dichromate for exactly 45 ml. of iron was calculated whenever the amount of the iron measured out varied slightly from that value.

Potassium dichromate was recrystallized, and part of this again recrystallized, to give the materials from which the A and B series of solutions were volumetrically prepared.

The A and B ferrous chloride solutions were prepared from about 2.8 grams of pure electrolytic iron (preserved in nitrogen), 25 ml. of distilled hydrochloric acid (density 1.10 at 25° C.), and water to bring to a volume of about 500 ml.

Stock solutions of about 20 liters each were made with ferric chloride and with potassium dichromate and were used in showing that no change occurred in the reagents, and what modifications caused error. In the former the hydrochloric acid content was equivalent to about 25 ml. of 1 to 1 acid per liter. However, the total acid present at the blue end point from all sources, and in all types of runs, varied no more than 10 per cent from the equivalent of the addition of exactly 14 ml. of hydrochloric acid with a density of exactly 1.10 (25° C.) in the method below, since variations were made in the addition of the acid to fit the particular type of run being made.

The stannous chloride was about 2 *N* and contained less than 100 ml. per liter of hydrochloric acid expressed as the 1 to 1 solution. The slowly dissolving tin metal used to keep it reduced did not cause appreciable introduction of impurities.

SILICOMOLYBDIC ACID INDICATOR. To 13.2 grams of Baker's reagent sodium silicate pentahydrate dissolved in water was added a solution made by dissolving 35 grams of Sterling "analysis certified" ammonium molybdate tetrahydrate and adding 49 ml. of concentrated sulfuric acid. The whole was made to a volume of 2 liters. (Molybdate is in less than equivalent amount, since the ratio of molybdenum to silicon is about 3 to 1 in place of the theoretical 12 to 1.)

N-PHENYLANTHRANILIC ACID INDICATOR. Eastman "white label" material (1.20 grams) was dissolved in a little warm water containing 0.72 gram of technical sodium carbonate, and the solution was made to 1200 ml. (*1*).

BLUE END POINT. The disappearance of the blue color at the first end point was determined by the following definition: Looking through the solution onto the surface of a horizontal daylight fluorescent lamp placed at the same height, the end point was considered as the last major change in shade, upon swirling after each drop of dichromate was added. With small samples this is synonymous with a change from blue to yellow or light green, and even with larger samples, at least during the day, a fairly good blue to green end point can be detected without the lamp. The last drops were added from 0.5 to 1 minute apart.

Determination of Iron

The determination of iron is described for application to volumetrically measured solutions of ferric chloride.

To 14 ml. of hydrochloric acid (density 1.10 at 25° C.) in a 500-ml. Erlenmeyer flask add 25 ml. of water. Boil until 5 to 7 ml. have been converted to steam, driving the air from the space above the solution past the water seal formed under a small watch glass placed over the mouth of the flask. Use a small sintered quartz boiling chip to obtain even boiling. (If much acid is present in the ferric chloride, proportionately less acid can be measured into the Erlenmeyer flask.) Transfer quickly from the burner first used to a hot plate regulated so that the solution boils only slowly, and add 45 ml. of the ferric chloride solution being analyzed. Upon resumption of boiling add 2 *N* stannous chloride drop by drop until the yellow color of the ferric ion disappears, and then add 5 drops of the silicomolybdic acid indicator to cause an intense blue in the solution. The watch glass is kept in place on the gently boiling solution as much as possible until the solution is cooled. Thus air is kept from re-entering the flask until cooling renders the solution practically invulnerable to air oxidation. For the 45 ml. of 0.1 *N* ferric chloride used by the authors, 2.75 ml. of stannous chloride were used, and at the blue end point the volume was close to 80 ml. If less than this amount of solution is to be analyzed, the volume at the first end point must be adjusted to 80 ml. by prior addition of water along with the ferric chloride.

Immediately titrate with standard potassium dichromate to the disappearance of the blue color. At once add a mixture of 85 ml. of water and 40 ml. of 1 to 1 sulfuric acid (density 1.49 at 25° C.), which has just been boiled for at least 3 minutes and is still hot. Boiling will start again almost at once. Heat for a total of 20 minutes to destroy the silicomolybdic acid, so it will not cause a muddy brown in place of a clear dark red color at the second end point. Remove from the hot plate and cool under running water without swirling (6 minutes were used in cooling).

Titrate with the dichromate with constant swirling, using a total of 8 drops of the *N*-phenylanthranilic acid indicator and observing the end point without use of the lamp mentioned above. The total volume at the end point was close to 200 ml. in the work of the authors, some evaporation occurring. The indicator goes

Table I. Titrations of Iron with Pure Dichromate

Iron Sample	Dichromate[a]		Blank to Be Subtracted	Calculated Normality of $FeCl_2$ or $FeCl_3$
			Ml.	
	B_1, 0.11743 *N*	B_2, 0.10134 *N*		
Stock $FeCl_3$	37.32	..	..	0.09728[b]
Pure $FeCl_2$, B_1, 0.09942 *N*	38.14	..	0.08	0.09932
		44.18[c]	0.09	0.09929
		0.58[d]		Av. 0.09931

[a] Ml. per 45.00 ml. of iron (per 0.50 ml. of iron in blanks). Normalities of dichromate by weight.
[b] Calculated from weight normality of pure iron, using ratio 37.32/38.14 (blanks canceling).
[c] Average of only 3 runs.
[d] Includes run with B_1 (0.47 ml.) equivalent to 0.55 ml. as B_2 as calculated from B_1/B_2 normality ratios.

Table II. Titrations of Stock $FeCl_3$[a] with Stock Dichromate

Type of Titration	Stock Dichromate[b]	Blanks 1A	Blanks 1B	Blanks 1C	Calculated Dichromate Normality[c]
Normal (by drops[d] with swirling)	43.55	0.56	0.54	0.55	0.10071
	43.54	0.54	0.55	0.53	0.10076
Fast addition of dichromate without swirling	43.57	..	..	..	0.10066
	43.57	..	..	..	0.10066
3 hours' boiling with sulfuric acid (watch glass in place)	43.55[e]	..	..	..	0.10071
	43.47[e]	..	..	..	0.10090
Same (no watch glass)	42.45[e]	..	..	..	0.10332
	42.59[e]	..	..	..	0.10298

[a] $FeCl_3$, 0.09733 *N*.
[b] Ml. per 45.00 ml. of $FeCl_3$ (per 0.50 ml. in blanks).
[c] −0.06-ml. blank on dichromate.
[d] Later it was considered normal procedure to add faster than by drops, but with continuous swirling, between two indicator changes.
[e] Represents 2 runs, averaged.

out of action rather easily, so that the 8 drops are added in installments. It seems likely that it precipitates, since the sodium salt of the indicator is used in the acidic solution. If the last addition of the indicator is close to the end point it must be followed by at least 2 separate drops of dichromate, the first of which does not cause appearance of the permanent red color.

It would seem wise to filter out any cloudy material, such as silica, during application of the method.

Should the blue end point be passed, another drop of stannous chloride can be added and the end point again approached.

Results

Solutions made from pure electrolytic iron whose normalities were 0.09942 and 0.09960 by weight were analyzed according to the method, using pure dichromate solutions. The normalities found were 0.09931 and 0.09953 after application of the blanks. Had the blanks not been applied the uncorrected volumes of dichromate would have been such that the higher values for the iron solutions would still check the known normalities to one part in a thousand. The use of blank runs is considered unnecessary unless the presence of appreciable impurities is suspected in the reagents. The results with pure ferrous chloride, B_1, solution are tabulated in Table I; similar results with pure ferrous chloride, A_1, pure dichromate, A_1, and pure dichromate, A_2, gave the second value discussed above.

In Tables I and II each figure represents an average of four runs, deviating from that average by not over 0.04 ml. except where otherwise noted. There is a 0.06-ml. deviation from the average in the case of the 42.45-ml. value for the "3-hour, no watch glass" work in Table II.

In Table I the stock ferric chloride was titrated with pure dichromate, B_1, which was also used to titrate pure iron, B_1. The normality of the former was thus found to be 0.09728 by using the pure iron, B_1, as the ultimate standard and employing the volume ratios of the dichromate. The normalities of the dichromate solutions, the impurities which might be present in the reagents, and any errors in the method all cancelled out in the above and in the similar untabulated comparison of the stock ferric chloride and the pure iron, A_1, solution. The latter gave 0.09738 *N* for the stock ferric chloride. In Table II the value 0.09733 was used for the latter solution and was obtained by averaging the two values given above. The normality of the stock dichromate was obtained from that of the stock ferric chloride (Table II).

Careful checks at the end of the work showed no appreciable change in the reagents and stock solutions during the course of the work.

In Table II the results with the stock solutions of dichromate and ferric chloride are arranged chronologically. The runs and check runs give an average of 0.10073 *N* for the stock dichromate for normal titrations against the 0.09733 *N* ferric chloride. The fast addition of nearly all the dichromate, in the presence of 4 of the 8 drops of indicator, did not materially alter the results when the titrations were finished with the remaining indicator in the normal manner (third and fourth lines of Table II).

Table II shows that there was very little error upon heating for 3 hours with the sulfuric acid unless the watch glass was not in place, when the error due to air oxidation[1] was such that even in 20 minutes it would have amounted to over 0.1 ml. In these experiments boiling was at a lower rate than usual in order to conserve solution volume.

Blank Runs

An elaborate system of blanks was used in the work tabulated in Table II, in order to test for any impurities present in the reagents. It was later concluded that blanks need not be made in routine application of the method.

The 1B blank runs actually used were made with but 3 drops of stannous chloride and with about 0.50 ml. of ferric chloride. This same type of blank was the only one needed in the work reported in Table I, since the ferrous chloride used there had oxidized but slightly and so needed but 3 drops of stannous chloride in the regular runs.

In the regular runs in Table II, 2.75 ml. of stannous chloride were used to reduce the ferric chloride, necessitating a blank with that amount of stannous chloride. These 1A blanks used up over 35 ml. of dichromate before the blue end point. Because of the large amount of green chromic ion present at this stage the total volume was made 130 ml. in place of the usual 80 ml. To test the effect of this volume change the 1C blanks were made in the same way as the 1B blanks, except that the volume at the blue end point was 130 ml., less water being used later, so as not to change the volume at the final end point from that in the method. In the 1A and 1C blanks the extra water was added with the approximately 0.50 ml. of iron solution. The volume effect was negligible, and the 1A blanks checked the other types.

In making the calculation of the actual correction to be made on the dichromate a case is selected from the first line of Table II, where 0.54 ml. of dichromate was needed for 0.50 ml. of ferric chloride in a 1B type blank. However, 45.00 ml. of ferric chloride required 43.55 ml. of dichromate, so 0.50 ml. should have required only 0.48 ml. of dichromate. The difference of 0.06 ml. is the blank to be subtracted from the volume of dichromate to give the 43.49 ml. actually used in titrating the reduced iron. The second line of Table II gives 0.07 ml. as the blank and the average is taken to be 0.06 ml. The blanks applied in Table I (next to last column) were similarly calculated and used.

Summary

A new method for the volumetric determination of iron uses silicomolybdic acid indicator in showing the beginning of ferrous ion oxidation. *N*-phenylanthranilic acid indicator

[1] Any loss of spray from uncovered flask is also included under this term.

shows the end of that oxidation with standard potassium dichromate solution. The volume of solution used between the two color changes is equivalent to the iron being determined.

Acknowledgment

Clarence J. Astill aided the senior author during the earlier preliminary work with the method.

The authors are indebted to H. H. Willard of the University of Michigan for the electrolytic iron.

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Contribution 63 from the Chemical Laboratories of the University of Utah.

Pressure-Regulating Apparatus for Vacuum Systems

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FREQUENTLY in practical laboratory work it is advantageous to employ a regulating device for vacuum systems in cases which do not warrant the expense or complication of a precision apparatus. Several vacuum regulators are described in the literature, representing a considerable range of complexity and precision. So far as is known none of these operate on a principle similar to that indicated below. The instrument described here is intended to combine simplicity with a moderate degree of precision. It is not difficult to construct or operate, is self-contained and positive in action, and will maintain a desired pressure in a reasonably tight system within ±0.2 mm. in the range 5 to 100 mm. Higher operating limits require modifications. Two types are illustrated; these differ only in the manner of controlling the operation point.

In operation, a high vacuum, chamber *A*, is allowed to act periodically, through a sintered-glass disk, on a system, *B*, in which it is desired to control the pressure. The operation is controlled by means of the mercury column in the open arm of a manostat, chambers *A* and *B* being alternately connected and sealed as the manostat fluctuates in response to pressure differences in *B*. The level difference, *h* (Figure 1), or the pressure, *p* (Figure 2), is then the operating pressure of the system.

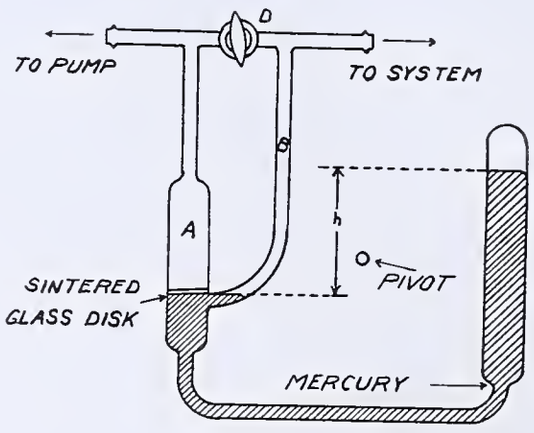

Figure 1

Figure 1 illustrates a unit in which the desired pressure is maintained by rotating the entire apparatus on a pivot until the porous disk is just sealed by one level of the mercury, as the difference in the levels between the two mercury columns equals the operating pressure. In practice this is not measured on the apparatus but is read on a manometer connected to *B*. An increase in pressure in *B* will result in a movement of mercury in the manostat tube, so as to open the disk between *A* and *B*. The stopcock, *D*, is not essential, but facilitates rapid evacuation of the system at the start. It is closed during operation.

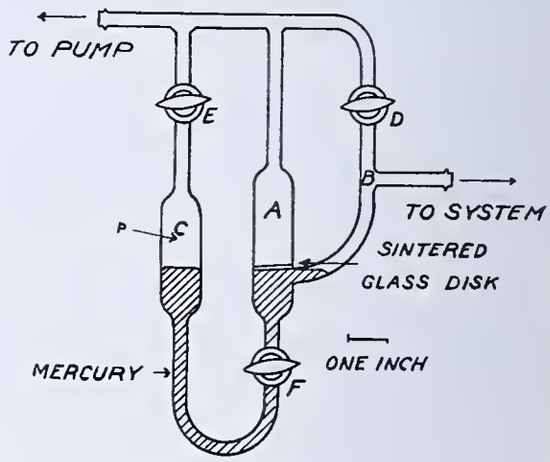

Figure 2

Figure 2 indicates a device employing a residual pressure in chamber *C*, rather than a balancing mercury column, to set the point at which the porous disk will be sealed by the manostat. The proper pressure is obtained in *C* by leaving open stopcocks *D* and *E* until the auxiliary manometer connected to *B* indicates the desired operation point, and then closing them. It is advisable to insert a stopcock (not shown) between the apparatus and pump to aid in this last adjustment; it should be closed as the pressure approaches the desired point to allow the pressure to become equalized. Further drop in pressure in *A* cannot affect the pressure in *B* unless a leak or gas evolution in *B* raises the pressure so as to open the sealed porous disk. Stopcock *F* is provided only to prevent rapid surges of mercury when air is allowed to enter the apparatus at the completion of an operation. Fine adjustments of the operating pressure are best made by slightly tilting the unit. A 0.75-inch disk constructed of 60-mesh glass or a commercial G-3 disk appears satisfactory for operation in the range 5 to 100 mm. Higher working pressures may be attained by using a finer sintered-glass partition than specified. Lower maximum operation pressure allows coarser disks, because of lower pressure differential across the disk. A satisfactory partition may be constructed of other porous materials cemented in position. Smooth operation is facilitated by mounting the disk in a slanting position.

The mercury placed in the manostat should be boiled out under vacuum, so that bubbles of gas will not rise in the closed arm of the manostat tube and cause a drift of operation pressure. The unit should be protected by a dry ice trap to avoid clogging of the disk. If uncondensed vapors pass through the trap an absorption tube may be added to the assembly.

Acknowledgment

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Comparative Tests of Chemical Glassware

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This paper describes the results of comparative tests of four brands of chemical glassware: Tamworth-Glasbake, Kimble No. N51a, chemical Pyrex, and Vycor (Corning Glass Works 96 per cent silica glass No. 790). The wares were compared with respect to resistance to acid, alkaline, and nearly neutral reagents, and resistance to thermal and mechanical shock. The chemical composition and the thermal expansivity of each glass were also determined.

THREE brands of borosilicate glass, varying somewhat in composition and general properties, are now produced in the United States in the form of beakers, flasks, and other apparatus for general laboratory use. These three wares are chemical Pyrex (Corning Glass Works No. 774), Kimble Glass Company's No. N51a, and Tamworth-Glasbake. (Tamworth-Glasbake is manufactured by the McKee Glass Co., Jeannette, Penna., and distributed by Tamworth Associates, Needham Heights, Mass. The authors are informed by the McKee Glass Co. that ware of the same composition will be distributed in certain parts of the country under the mark "Glasbake").

A series of tests designed to determine the relative resistance to chemical attack of these three wares has been made at the National Bureau of Standards, similar to tests made about 25 years ago (*5*) when the European wares commonly used up to that time began to be displaced by several products of American manufacturers. The chemical compositions of the glasses and their thermal coefficients of expansion were also determined, and some information was gained concerning their resistance to mechanical and thermal shock.

A new type of chemical glassware sold under the trademark Vycor (Corning Glass Works' 96 per cent silica glass No. 790) was included in some of the tests.

Erlenmeyer flasks of 250-ml. capacity were used for nearly all the tests described in this report. The factories where the different glasses are produced were visited by one of the authors to see the flasks made (except the Vycor) and to earmark them for delivery to the bureau. At the same time samples of the glasses in the form of rods were prepared and brought to the bureau. These samples, together with some in the form of slabs, were used for some of the measurements of the thermal expansivity.

Chemical Composition

The major constituents of the glasses tested were determined by routine chemical analyses (by Francis W. Glaze). The results obtained, as given in Table I, are intended to show the approximate composition, except with respect to arsenic and antimony. These elements were determined with greater care (by J. A. Scherrer).

Thermal Expansivity

The average coefficient of linear expansion in the range 20° to 300° C. was determined (by Peter Hidnert) with the apparatus described by Souder and Hidnert (*4*) on specimens of the rods obtained for that purpose. The coefficient was also determined (by L. H. Maxwell and J. B. Saunders) by the interferometer method described by Peters and Cragoe (*2*), as amplified by Saunders (*3*), on specimens cut from slabs and from flasks.

The rods were tested first "as received"—i. e., air-cooled or unannealed—and again after the same specimens were carefully annealed. Annealing consisted in heating the rods to 590° C. for 5 hours, cooling to 515° C. at the rate of 8° C. per hour, holding at the latter temperature for 48 hours, and then cooling slowly to room temperature. The initial cooling rate from 515° C. was about 1° C. per hour and was gradually increased to a maximum of 10° C. per hour as the temperature fell. (It happened to be convenient to anneal the rods in the same furnace with some optical glass; hence this unnecessarily long schedule.)

The results of the expansivity measurements are given in Table II. The data show that the two methods of measurement give practically identical results, unless there were compensating differences in the samples and methods used.

TABLE I. COMPOSITION OF SAMPLES

	Glasbake %	Kimble %	Pyrex %	Vycor[a] %
SiO_2	78.4	74.7	81.0	96.3
B_2O_3	14.0	9.6	13.0	2.9
R_2O_3[b]	2.5	5.6	2.2	0.4
ZnO	N. d.[c]	0.1	N. d.	N. d.
CaO	0.1	0.9	Neg.[d]	Neg.
BaO	N. d.	2.2	N. d.	N. d.
MgO	Neg.	Neg.	N. d.	N. d.
Na_2O	5.0	6.4	3.6	<0.02
K_2O	Neg.	0.5	0.2	<0.02
As_2O_3	0.037	0.027	0.002	0.005
Sb_2O_3	0.038	0.009	N. d.	N. d.

[a] 0.3 per cent of undetermined constituents.
[b] Chiefly Al_2O_3.
[c] Corresponding constituent was not detected.
[d] Negligible amount of constituent.

TABLE II. AVERAGE COEFFICIENT OF LINEAR EXPANSION × 10^6, BETWEEN 20° AND 300° C.

Condition	Brand of Glassware: Glasbake	Kimble	Pyrex	Vycor
As received	3.7(H)[a]	4.9(H)	3.3(H)	0.8(S)
As received	3.7(M)	4.9(M)	3.3(M)	....
Annealed	3.4(H)	4.8(H)	3.0(H)	....
Annealed	3.6(M)	4.9(M)	3.1(M)	....

[a] (H) values were obtained by P. Hidnert, (M) by L. H. Maxwell, and (S) by J. B. Saunders.

Relative Chemical Resistance

In studying the effect of various aqueous solutions and other reagents on the glasses, no attempt was made to determine the nature of the reactions—that is, to find out what changes occurred at the surface of the glass or whether the material removed was of the same composition as the glass. An exception to this general rule was made in determining the amount of arsenic removed by alkaline solutions, since this is of known interest in certain uses of chemical glassware, such as in the determination of arsenic in insecticide residues on fruits.

In general it appeared that the analyst's interest would lie in the total amount of substance removed from the glass, in relation both to the contamination of solutions undergoing analysis and to the relative rates at which the wares would become unserviceable through roughening of the surface.

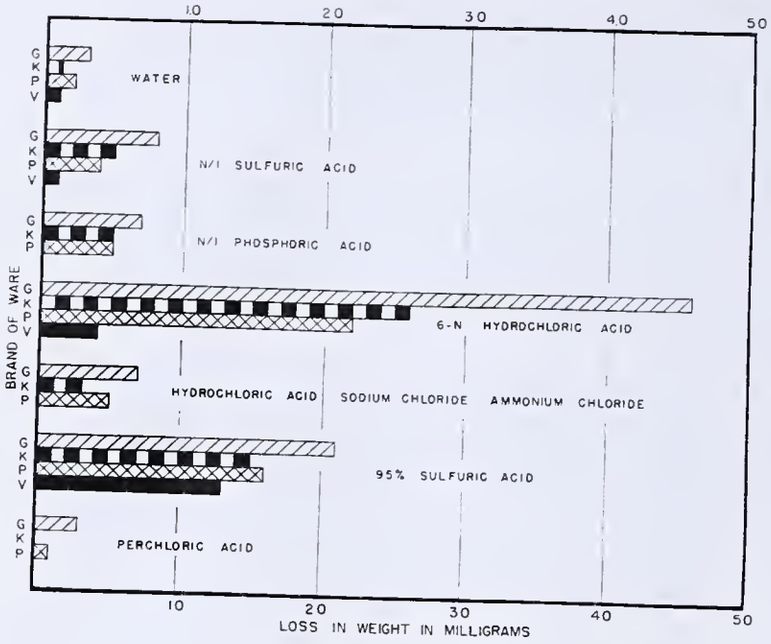

FIGURE 1. COMPARATIVE RESISTANCE TO ACID REAGENTS AND WATER

Average loss in weight in milligrams per flask per 6-hour period of exposure

Because precipitates tend to adhere to etched glass surfaces so tenaciously as to make quantitative transfers difficult or impossible, the useful life of much chemical glassware may be ended by excessive etching or scratching of the inner surfaces, rather than by breakage.

Since the chemist cannot readily have glassware made to order (with respect to composition or properties) but must necessarily make a choice among products that are commercially available, it seemed useless to attempt to devise tests that might yield results which would be independently reproducible and which might have some absolute significance. Instead, the methods of testing chemical resistance were planned solely to yield comparative results. The three brands (all except the Vycor) were always treated simultaneously, thus making it unnecessary to define the conditions of treatment very closely. All the tests of chemical resistance were made on samples taken from a single lot of 250-ml. Erlenmeyer flasks of each type of ware. It is possible that similar tests, made on other lots of the wares and in other laboratories, would not yield the same numerical results, but it is believed that, barring significant changes of composition in any of the wares, similar tests would give the same relative results.

All the flasks used were numbered for identification. They were prepared for testing by gentle scouring, inside and out, with a pumice soap, thorough rinsing with distilled water, heating overnight in an electrically heated oven at about 110° C., and weighing. Prior to weighing they were placed near the balance in a covered box for several hours and then placed, three at a time (together with a tare flask) within the balance case for at least half an hour. One flask of each type, similarly treated, was used as the tare. The tare flasks were rinsed and dried before each set of weighings and were scoured only between tests with different reagents.

By taking pains in handling the flasks and in protecting the air of the balance case from sudden changes in temperature or humidity, the observed weights were reproducible, with rare exceptions, within 0.1 or 0.2 mg. Losses in weight caused by scouring and rinsing the flasks were negligible. These conclusions were reached from a series of five repeated weighings of nine flasks (three each of Glasbake, Kimble, and Pyrex) not treated except by cleaning and drying. In this series the tare flasks were not scoured, but only rinsed and dried whenever the others were scoured, rinsed, and dried.

With a few exceptions, which will be indicated, 200 ml. of the selected reagent were placed in each of three flasks of each of the wares, which were heated simultaneously on an electric hot plate. Gentle boiling was maintained for 6 hours. During the treatment each flask was closed with a funnel-shaped cover, made of the same glass, to serve as a partial condenser. Flattened pellets of gold sponge served admirably to prevent bumping and were not attacked by any of the reagents except perchloric acid, which did attack them slightly. About midway of the 6-hour period of boiling the volume of reagents was restored to about 200 ml. by replenishing with hot water, except as otherwise noted. The flasks made of the different glasses were so nearly of the same shape and capacity that variations in the surface exposed to attack were regarded as negligible. Because they were exposed to condensing vapors and to spray from the boiling liquid, the glass surfaces above the level of the reagent were also attacked, although probably not equally with the submerged portions.

After the 6-hour treatment, the flasks were emptied, rinsed with water, gently scrubbed inside and out, then thoroughly rinsed again, and heated in the oven overnight at about 110° C. The weighing procedure after treatment was the same as before. The same flasks were then subjected to a second treatment for 6 hours, and in some instances to a third.

The Vycor flasks were not received until after the tests had been started. The tests of this ware were, therefore, not made simultaneously with those of any of the other three brands. However, since the conditions of test were duplicated rather closely, it is believed that the results can be safely compared with those for the other wares.

The reagents used, besides distilled water, can be divided into three groups: strongly acid, strongly alkaline, and nearly neutral. For convenience the results obtained with water are included with those of the acid group. In order to show the extent of the variations in results obtained with different flasks and during the successive periods of test the individual results will be published in detail (*6*). In the present paper, the average loss per flask, per 6-hour period, is shown in Figures 1, 2, and 3.

Reagents and Conditions of Test

ACID REAGENTS AND WATER.

1. Distilled water; 3 periods.
2. Approximately 1 *N* sulfuric acid; 3 periods, 6 flasks.
3. Approximately 1 *N* phosphoric acid (0.33 molar); 2 periods.
4. Approximately 6 *N* (constant boiling) hydrochloric acid. About midway of the 6-hour period of boiling the flasks were replenished with acid of the same strength instead of with water; 2 periods.
5. A solution containing 50 ml. of concentrated hydrochloric acid, 50 grams of sodium chloride, and 50 grams of ammonium chloride in 1 liter; two periods.
6. Sulfuric acid, 95 per cent. Fifty milliliters were used instead of the usual 200 ml. and the flasks were heated on a gas hot plate. Since the area of glass exposed to the boiling acid was not the same as with most of the other reagents, the results are not directly comparable with those obtained with the others. No replenishment of acid was necessary; three periods.
7. Perchloric acid. Fifty milliliters of 60 per cent acid were used for the first period of 6 hours. The electric hot plate used did not supply enough heat to keep the acid boiling, but much of the acid evaporated. For the second period 100 ml. of acid were used. The flasks were heated on a gas hot plate to keep the acid boiling gently. No acid was added during either of the 6-hour periods; two periods.

ALKALINE REAGENTS (two 6-hour periods for each reagent).

1. 0.05 *N* sodium hydroxide.
2. 0.5 *N* sodium hydroxide.
3. 0.5 *N* potassium hydroxide. Arsenic was determined (by J. A. Scherrer) in the potassium hydroxide solution taken from two of each of the three brands of flasks after the first 6-hour period of attack. The results, in milligrams of arsenic removed

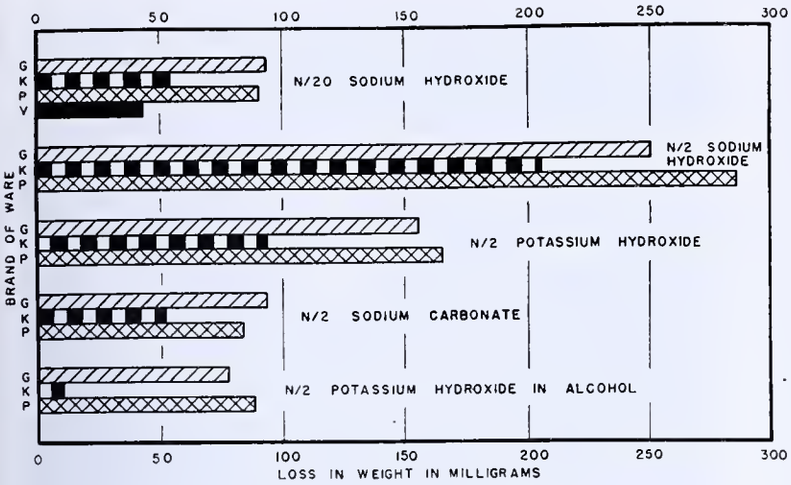

FIGURE 2. COMPARATIVE RESISTANCE TO ALKALINE REAGENTS
Average loss in weight in milligrams per flask per 6-hour period of exposure

from each flask, are: Glasbake, 0.14, 0.16; Kimble, 0.06, 0.07; Pyrex, not detected (less than 0.004 mg.).

4. 0.5 *N* sodium carbonate.

5. 0.5 *N* potassium hydroxide in 95 per cent alcohol. When this solution was used the flasks were connected with water-cooled reflux condensers by means of rubber stoppers. The first period was 1 hour and the second 2 hours instead of the usual two 6-hour periods. The object of this departure from the usual procedure was to simulate actual conditions under which alcoholic potash solutions are frequently used. In Figure 2 the average losses per hour are multiplied by six in order to make the results comparable with those obtained with the other reagents.

6. A solution containing, at the outset, 100 ml. of ammonium hydroxide (about 28 per cent ammonia) and 100 grams of ammonium chloride in 1 liter. This solution was boiled in the usual way, and was replenished with water. These results are not shown in Figure 2. The average losses per flask, per 6-hour period, were: Glasbake, 2.1 mg.; Kimble, 1.1 mg.; Pyrex, 1.4 mg.

7. Ammonium hydroxide (about 28 per cent ammonia). This reagent was used at room temperature. The flasks were closed with rubber stoppers. The first period was 8 days and the second 34 days. These results are not shown in Figure 2. The average losses per flask for the two periods were: Glasbake, 3.2 mg.; Kimble, 1.9 mg.; Pyrex, 1.9 mg.

NEARLY NEUTRAL REAGENTS. This group of reagents was made up chiefly of 5 per cent solutions of sodium chloride (actually 50 grams of sodium chloride in 1 liter of solution) adjusted to selected points on the pH scale by means of mixtures of potassium dihydrogen phosphate and disodium hydrogen phosphate. The effect of nearly neutral salt solutions was studied in some detail after it was observed that an unbuffered 5 per cent solution of sodium chloride caused a pronounced attack of all the glasses (although in varying degree). The indicated pH is that of the solution at the beginning of the test period, at room temperature. The approximately neutral solutions used were as follows:

1. Sodium chloride, 5 per cent solution, not buffered. The solution was slightly acid at the beginning of the test period but the contents of the flasks were slightly alkaline at the end of the period, especially in the group which showed the greatest attack; three 6-hour periods.

2. Sodium chloride, 5 per cent solution in 0.001 *N* hydrochloric acid. All the flasks were so slightly attacked by this reagent that they were used again with the next one. This is the only instance in which any flasks were used for tests with more than one reagent; two 6-hour periods for this and all succeeding tests.

3. Sodium chloride, 5 per cent solution, buffered at pH 6.2. This solution contained, in addition to the sodium chloride, 10.85 grams of disodium hydrogen phosphate dodecahydrate and 4.55 grams of potassium dihydrogen phosphate in 1 liter.

4. Sodium chloride, 5 per cent solution, buffered at pH 7.0. In addition to the sodium chloride, the solution contained 19.0 grams of disodium hydrogen phosphate dodecahydrate and 1.15 grams of potassium dihydrogen phosphate in 1 liter.

5. Sodium chloride, 5 per cent solution, buffered at pH 7.7. The buffer-salt concentrations were 33 grams of disodium hydrogen phosphate dodecahydrate and 0.25 gram of potassium dihydrogen phosphate in 1 liter.

6. Sodium chloride, 5 per cent solution, buffered at pH 8.4. The buffer salt concentration was 33 grams of disodium hydrogen phosphate dodecahydrate in 1 liter.

7. Buffer solution, at pH 6.8, without sodium chloride. The solution contained 10.85 grams of disodium hydrogen phosphate dodecahydrate and 4.55 grams of potassium dihydrogen phosphate in 1 liter. The results may be compared with those of Nos. 3 and 4 to show the marked effect of sodium chloride.

8. Potassium chloride, 7 per cent solution (70 grams in 1 liter), buffered at pH 7.2. The buffer salt concentrations were 18.5 grams of disodium hydrogen phosphate dodecahydrate and 1.15 grams of potassium dihydrogen phosphate in 1 liter. This solution contained approximately the same molar concentrations of salts as No. 4 and was at nearly the same pH. The two sets of results show a marked difference between the effect of sodium and potassium chloride on the three glasses.

Resistance to Mechanical Shock

The resistance of the 250-ml. flasks to mechanical shock was determined (by Donald Hubbard) by the method currently used for determining the resistance of chinaware to chipping.

Briefly, this consisted in placing a flask firmly between two cast-iron blocks forming a 90° V and striking the flask at its maximum diameter with the tup of a pendulum of fixed length, swinging through arcs of increasing length until the glass broke. (A complete description of this apparatus and its method of application is given in Federal Specification for Chinaware, *1*.) After each impact, the flask was rotated slightly so that successive blows were delivered at different points on the glass. The energy (in foot-pounds) required to break the glass was computed from the weight of the tup and the distance through which it swung. Vycor flasks were not included in these tests.

The results obtained on 12 flasks of each kind are as follows: Glasbake, average weight 90.6 grams impact energy 0.20 to 0.65 foot-pound, average 0.44 foot-pound; Kimble, average weight 62.2 grams, impact energy 0.10 to 0.22 foot-pound, average 0.15 foot-pound; Pyrex, average weight 80.0 grams, impact energy 0.20 to 0.45 foot-pound, average 0.29 foot-pound. The average results indicate significant differences in resistance to mechanical shock. In this respect the

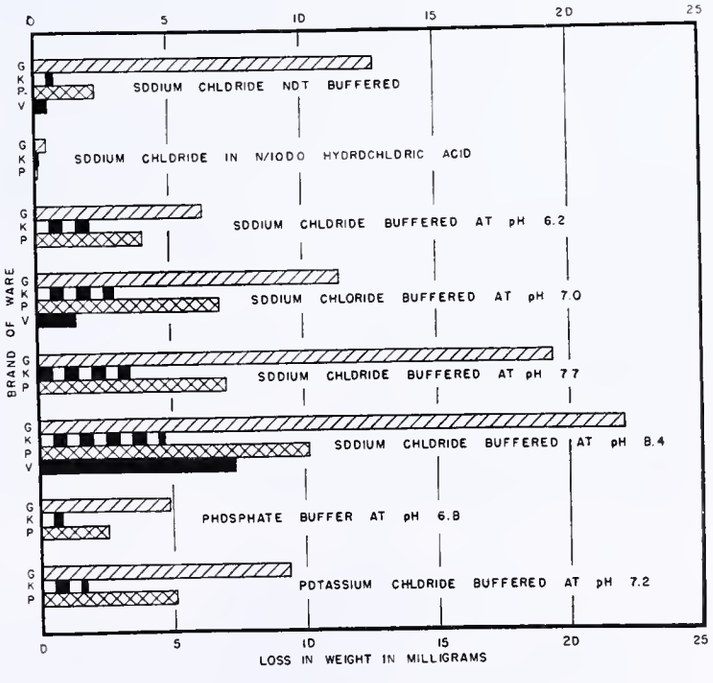
0 5 10 15 20 25
SODIUM CHLORIDE NOT BUFFERED
SODIUM CHLORIDE IN N/100 HYDROCHLORIC ACID
SODIUM CHLORIDE BUFFERED AT pH 6.2
SODIUM CHLORIDE BUFFERED AT pH 7.0
SODIUM CHLORIDE BUFFERED AT pH 7.7
SODIUM CHLORIDE BUFFERED AT pH 8.4
PHOSPHATE BUFFER AT pH 6.8
POTASSIUM CHLORIDE BUFFERED AT pH 7.2
G K P V
G K P
G K P
G K P
G K P V
G K P
G K P
BRAND OF WARE
LOSS IN WEIGHT IN MILLIGRAMS

FIGURE 3. COMPARATIVE RESISTANCE TO NEARLY NEUTRAL REAGENTS
Average loss in weight in milligrams per flask per 6-hour period of exposure

three wares fall in the order of their relative weights. However, there is a wide range in the individual values in each group, probably because of variations in thickness and in the amount of surface checking and scratching incident to handling.

Table III. Resistance to Thermal Shock

Temperature ° C.	Number of Breaks Glasbake Group A	Glasbake Group B	Kimble Group A	Kimble Group B	Pyrex Group A	Pyrex Group B
175	0	1	0	5[a]	0	0
200	3	4	2	7	0	0
225	3	6	6	2	0	4
250	4	5	7	2	2	6
275	5	2	3	2	3	2
300	3	..	..	..	10	4
325	..	..	..	..	3	2
Average breaking temperature	253	230	240	210	294	267

[a] The relatively large number of this group which broke at 175° suggested that a lower starting temperature should have been used. Accordingly another set of 18 flasks was tested under the same conditions except with a starting temperature of 125° C. None of this group broke at 125° or 150° and the average breaking temperature was 238° C.

Resistance to Thermal Shock

Resistance to thermal shock was determined (by Donald Hubbard) by slowly heating a flask, containing 100 ml. of a high-flash mineral oil, on a hot plate to a selected temperature and then quickly immersing it to the neck in ice water. If the flask did not break it was heated to a higher temperature and again suddenly chilled. This was repeated with increments of 25° C. until the flask cracked.

Early in the tests it was noted that the average breaking temperature was higher for flasks which had been carefully removed from their wrappings and tested directly than for flasks which had been handled somewhat, in about the way glassware is commonly handled from the time it leaves the storeroom shelf until it has been cleaned and otherwise made ready for its first service in the laboratory. This handling involved relatively gentle contact of the flasks with one another and with the laboratory bench, which undoubtedly causes minute surface injuries, usually too slight to be visible. Accordingly, tests were made of two groups of eighteen flasks of each brand (except Vycor). One group was tested directly as removed from the shipping containers and the other after normal handling. The results are given in Table III.

The results obtained with Group A, flasks taken directly from their shipping containers, are probably of less significance than the others, since they represent ware in a condition which cannot be maintained in service, and since they may reflect differences in the care with which the flasks were packed for shipment. On the other hand, the results of Group B cannot be safely regarded as representing the average condition of ware in service, since no attempt was made to learn whether the average breaking temperature decreased with prolonged or rougher handling.

Vycor ware was not included in these tests. Its very great resistance to thermal shock was demonstrated by heating a flask to redness and cooling it under a water tap, without its breaking.

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Steam-Distillation of Small Quantities of Volatile Oils Lighter than Water

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THE method generally used to determine the amount of steam-distillable oil in a sample is to collect the distillate in a graduated cylinder or in a separatory funnel. In the former case, the volume of oil may, if sufficient is present, be read directly; in the latter case it may be read after running off the water and collecting the oil in a graduated cylinder. Both methods are subject to serious error when only 1 or 2 ml. of oil are distilled over. Loss occurs because the oil sticks to the sides of the cylinder or funnel. A graduated cylinder of convenient size to collect the water distilled over has too large a surface area to measure the oil accurately in such small amounts.

Figures 1 and 2 illustrate how these defects can be eliminated by a simple combination of a graduated tube and separatory funnel.

Figure 1

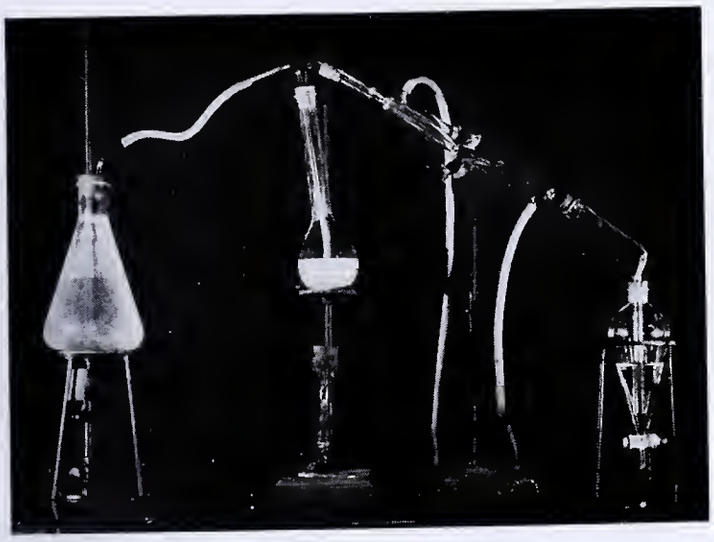

FIGURE 2

Insert a graduated tube, conveniently a part of a broken buret, open at both ends, in the neck of the separatory funnel by means of a cork which has a slit to permit air to escape. The tube should reach nearly to the bottom of the funnel and sufficient water be added to cover the bottom opening. The adaptor attached to the condenser should lead into the top of the graduated tube.

Distill in the usual manner. As volatile oils lighter than water float on the surface, the oil distilled over will always remain in the graduated tube and the water will rise in the funnel. As water is collected, remove it from the funnel by opening the stopcock, taking care that the water level is never below the bottom of the graduated tube. Continue the distillation until the oil is all distilled over. This point is easily found, as the volume of oil can be read at any time during the distillation. Finally, read the volume of oil in the tube, drain off the water completely, and collect the oil, which is now in the separatory funnel, for further examination.

Hydrolysis and Catalytic Oxidation of Cellulosic Materials

A Method for Continuous Estimation of Free Glucose

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Mellon Institute, Pittsburgh, Penna.

THERE are few direct methods available for the investigation of the submicroscopic structure of cellulose and, as a result, the elaboration of essential information on the natural fibers and industrial cellulosic products has been retarded. X-ray methods are restricted to the crystalloid fraction, and copper reduction tests indicate relative amounts of chemical degradation at the molecular level. Dispersions of cellulose, such as in cuprammonium hydroxide, reflect the properties of the dispersed unit rather than the antecedent structure. In this paper a new method of investigation is presented and discussed, based upon the observation that quantitative hydrolytic breakdown rates of cellulose in solutions of acid can be estimated continuously and interpreted in terms of structure.

Several recent articles have dealt with the evolution of carbon dioxide from simple sugars, polysaccharides, uronic acids, and mixtures of these substances (*3*, *5*, *6*, *7*). Whistler, Martin, and Harris (*7*) conducted the digestion of glucose and polysaccharides for 8 hours in boiling 12 per cent hydrochloric acid under an atmosphere of nitrogen, obtaining yields of about 0.38 per cent carbon dioxide from pure glucose and about 0.17 per cent from purified, pectate-free cotton cellulose. They showed, also, that with proper control the rates are reproducible. Nickerson and Leape (*5*) used air as the carrier gas and observed small and fairly uniform amounts of carbon dioxide from purified cottons of different varieties.

Various other cellulosic materials have been examined. According to Whistler and co-workers (*7*), glucose, viscose and cuprammonium rayons, and cellulose acetate corrected for its acetyl content yield carbon dioxide at similar, constant rates; purified cotton, however, is characterized by a much lower rate. These carbon dioxide data were reported as percentages based on the weight of starting material. Whistler's data could have been calculated in terms of moles rather than unit weights. As the capacity of glucose and its polymers to yield carbon dioxide must depend primarily on certain carbon-oxygen linkages of the glucose residue, it is apparent that equal weights of glucose and anhydroglucose (cellulose) would not represent comparable units; a unit weight of cellulose contains about 1.11 weights of glucose. Thus Whistler's figures for glucose, the regenerated celluloses, and the cellulose fraction of cellulose acetate appear to be similar, but, on the basis of equivalent weights, glucose would exhibit a 10 per cent greater rate of evolution than the regenerated and substituted celluloses.

The somewhat smaller, true evolution of carbon dioxide from cellulosic materials suggested that at least two reactions might be involved. The first reaction might be a rapid hydrolysis to glucose; the second, a slower oxidation of the glucose to an unstable intermediate product which decomposes and yields carbon dioxide. If, then, the second step could be accelerated and kept in pace with the hydrolysis, carbon dioxide evolution rates might provide data on the breakdown rates of celluloses. A method based upon this mechanism has been evolved.

It was necessary as a first step to determine whether or not the carbon dioxide output from glucose could be increased and controlled. Preliminary experiments indicated that 9 per cent hydrochloric acid would be more satisfactory for the present investigation than 12 per cent. Other exploratory work led to the temporary selection of 0.5 molar as a suitable concentration of salt catalysts. The reasons for these choices are discussed in the subsequent sections.

Catalyst

It appeared that the oxidative process would be simplified if complicating oxygen-donator groups, such as chromates and sulfates, were avoided. Accordingly, the study of catalysts was restricted to the chlorides of metals, which were used at 0.5 *M* concentration in 9 per cent hydrochloric acid with pure glucose (Merck's C. P. anhydrous dextrose) as the oxidizable substance. The catalytic activities of several salts are given in Table I.

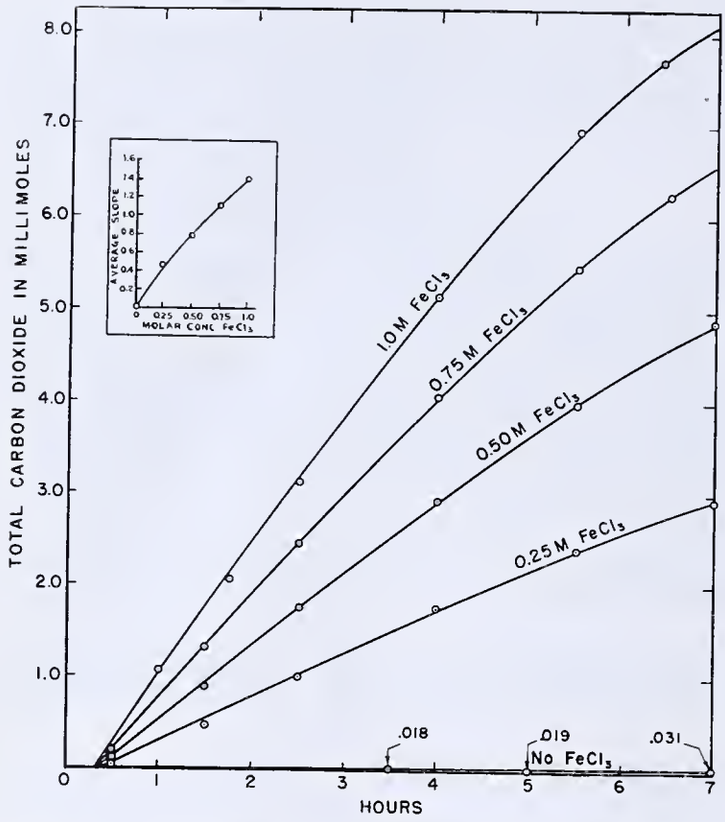

FIGURE 1. CATALYTIC AND CONCENTRATION EFFECTS OF FERRIC CHLORIDE ON EVOLUTION OF CARBON DIOXIDE FROM GLUCOSE

1 gram of glucose dissolved in 150 ml. of 9% hydrochloric acid solution. HCl concentration 9.0% throughout.

These data show clearly that ferric chloride increases the relative carbon dioxide output from glucose more than two hundred fold; consequently, ferric chloride was chosen as a suitable catalyst for the reaction.

The catalytic effects of ferric chloride in 9 per cent hydrochloric acid on the oxidation of glucose were determined at different concentration levels. The results, plotted in Figure 1, indicate that the carbon dioxide evolution curves are not independent of ferric chloride concentration. The approximate slopes of the curves—i. e., carbon dioxide evolution rates—appear to be almost directly proportional to ferric chloride concentration (Figure 1, inset).

TABLE I. EFFECT OF METAL CHLORIDES IN 9 PER CENT HYDROCHLORIC ACID ON EVOLUTION OF CARBON DIOXIDE FROM GLUCOSE

Salt	Salt Concentration *M*	Carbon Dioxide per Mole of Glucose in 5 Hours *Mole*
$FeCl_3$	0.5	0.65
$CuCl_2$	0.5	0.29
$SnCl_4$	0.5	0.016
$NiCl_2$	0.5	0.007
None	0	0.003

Hydrochloric Acid

The selection of 9 per cent hydrochloric acid for the work just described was based on indirect, preliminary experiments. Results of a more systematic investigation are shown in Figure 2; in the latter experiments ferric chloride and glucose concentrations were held constant while acid concentration was varied. With the exception of that for unsupplemented ferric chloride, the curves illustrate the dependence of reaction velocity upon acid concentration. (When no acid is added, the boiling ferric chloride–glucose solution is at first very turbid and rust colored but becomes clear again after a few hours of refluxing.) If the approximate slopes of these curves are plotted against the corresponding acid concentrations (Figure 2, inset), an inflection can be discerned in the region of 9 per cent acid. Below this concentration the curves, convex downward in the first few hours, gradually approach maximum slopes; at higher concentrations the initial maximum slopes decrease and, after a few hours, the curves become convex upward. Furthermore, at the high acid concentrations hot solutions of glucose become discolored and form a dark brown precipitate in increasing quantity. A possible interpretation of these results is that, under the conditions indicated, glucose or its products become activated and polymerize. When the polymer precipitates, reactant is lost and the carbon dioxide evolution velocity decreases.

Eight per cent hydrochloric acid was selected as the most consistent concentration for the purpose. A compromise between antagonistic effects, it is below the range of precipitate formation and, at the same time, high enough to reduce the initial curvature. Thus this concentration of acid gives a carbon dioxide–time curve that is approximately linear for a considerable digestion interval.

The dependence of carbon dioxide evolution rate on both ferric chloride and hydrochloric acid concentrations made a stock solution desirable. A sufficient volume, containing 8 per cent hydrochloric acid and 0.6 mole of ferric chloride per liter, was prepared for subsequent experiments. The reasons for this amount of acid have been discussed. The choice of the ferric chloride concentration was somewhat arbitrary; a rapid evolution of carbon dioxide would decrease experimental errors, but an extremely rapid rate might exceed the safe absorbing capacity of the apparatus.

Glucose Concentration

The experiments outlined above were conducted at a constant glucose concentration. Because the hydrolysis of cellulose would represent the continuous addition of free

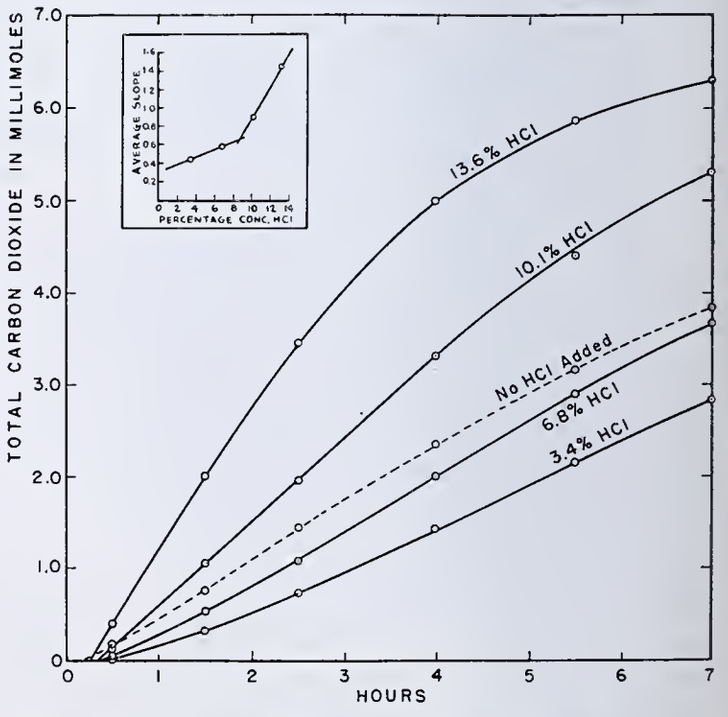

FIGURE 2. INFLUENCE OF CONCENTRATION OF HYDROCHLORIC ACID ON LIBERATION OF CARBON DIOXIDE FROM GLUCOSE

1 gram of glucose dissolved in 150 ml. of 0.5 *M* ferric chloride solution. $FeCl_3$ concentration 0.5 molar throughout.

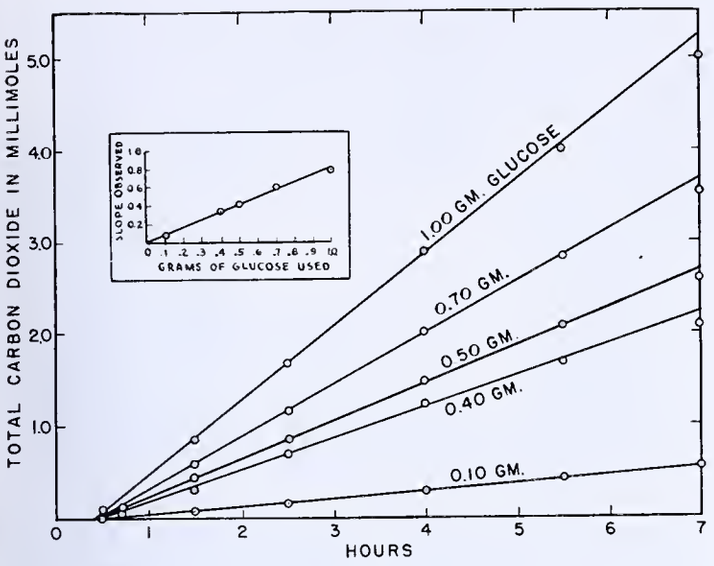

FIGURE 3. RATES OF EVOLUTION OF CARBON DIOXIDE

From varying amounts of glucose in 150 ml. of 9% hydrochloric acid–0.5 *M* ferric chloride solution.

glucose to the system, it was necessary to determine the effects of varying the glucose concentration when the acid and catalyst concentrations were held constant. The results, presented in Figure 3, are based on equal volumes of acid-catalyst solution and the specified amounts of anhydrous, crystalline glucose.

The curves are linear and, within a small error, the slopes are directly proportional to glucose concentration. But the evolution of carbon dioxide does not begin instantaneously. Time is reckoned from the onset of boiling in the solution and it is evident (Figure 3) that there is an induction period of about 0.4 hour before carbon dioxide appears in quantity.

This induction period may be the time required for appreciable carboxyl formation and decarboxylation to occur. That the same lag is present even after the digestion has been in progress for some time is demonstrated in Figure 4, which shows the effect of a second addition of glucose. The lag occurs in both instances. This phenomenon suggests that the induction period is not a peculiarity associated with the commencement of the experiment but intervenes between any addition of glucose and the appearance of carbon dioxide. Therefore the rate of carbon dioxide evolution at any time may be taken as a measure of the amount of glucose present in the system 0.4 hour previously.

To summarize, hydrochloric acid and ferric chloride in the proper concentrations cause glucose to yield carbon dioxide at a rapid but fairly constant rate; the rate of evolution is roughly proportional to the glucose concentration; there is a uniform time lag between additions of glucose to the system and the appearance of carbon dioxide.

Apparatus and Method

The apparatus of Dickson, Otterson, and Link (*4*) for uronide carbon dioxide was modified to facilitate rate measurements. A schematic diagram of the complete setup is shown in Figure 5. The gravimetric type described by Whistler and co-workers (*7*) is probably also suitable for the purpose.

The present modification includes a flowmeter, *A*, a sulfuric acid trap, *B*, to remove volatile carbonaceous products (*3*, *7*), a thermometer in the reaction flask, parallel interchangeable carbon dioxide absorption columns, *H*, *H'*, a bubble counter, *C*, and a soda-lime tower, *D*.

The operation of this apparatus differs from the method of Dickson and co-workers only in the use of the parallel columns. The carrier air stream is diverted to either branch of the absorption system by means of a 3-way stopcock, *E;* the other stopcocks in the receiving branch, *F* and *I*, are turned to the proper positions. *I* and *I'* are small needle valves.

The substitution of columns involves some preliminary manipulations. The second column is assembled, set in place, and swept out for a few minutes with carbon dioxide–free air from the auxiliary scrubbing train, *C*, *D*. While this air current is still flowing, the barium hydroxide solution in the dropping funnel is admitted slowly to the column and the final volume is adjusted with successive rinses of boiled distilled water. The auxiliary air stream is then interrupted by a turn of stopcock *F'* which leaves the branch ready to receive the carbon dioxide carrier gas.

After the diversion of the carrier gas to the second branch, *F'*, *G'*, *H'*, any residual carbon dioxide in the tubing and flask, *H*, is drawn into the column by means of the auxiliary air input. This column is then isolated with stopcocks *F* and *I* removed

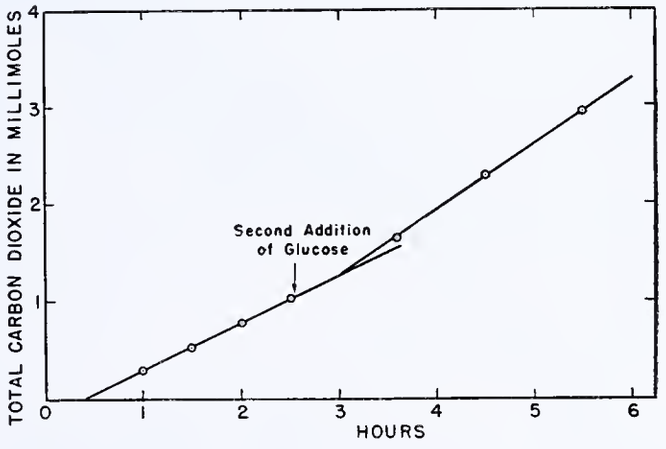

FIGURE 4. INDUCTION PERIOD BETWEEN ADDITION OF GLUCOSE AND APPEARANCE OF CARBON DIOXIDE SHOWN TO OCCUR BOTH AT BEGINNING AND DURING A RUN

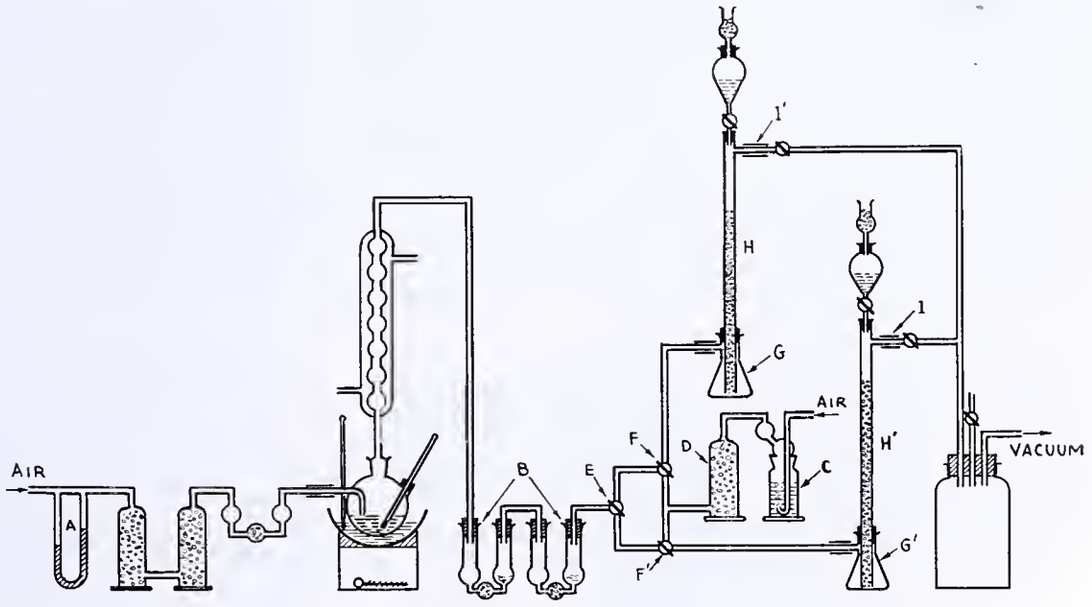

FIGURE 5. SCHEMATIC DIAGRAM OF APPARATUS FOR CARBON DIOXIDE RATE MEASUREMENTS

and the titration is made in the usual way. Hollow 3-ml. glass beads are satisfactory for the columns. A bulb-driven wash bottle tends to avoid error from the accumulation of respiratory carbon dioxide.

A 150-ml. volume of acid-catalyst solution appears to be a satisfactory amount for a single experiment. This volume of the stock solution was used in the blank on the apparatus and reagents and in the series of runs plotted in Figure 3. From the latter results it is evident that the rates of carbon dioxide evolution are not dependent upon the ratio of glucose to solution volume in the range specified. A rough measurement of the volume of acid-catalyst solution is, therefore, sufficiently accurate.

The weight of sample is chosen so that the total amount of free glucose acquired by the system is less than 1 gram. The rates of evolution then fall within the range mentioned above and the safe absorbing capacity of the apparatus is not exceeded. Two-gram samples of the natural celluloses and 1-gram samples of the regenerated and substituted celluloses have been found to be within the suggested limit.

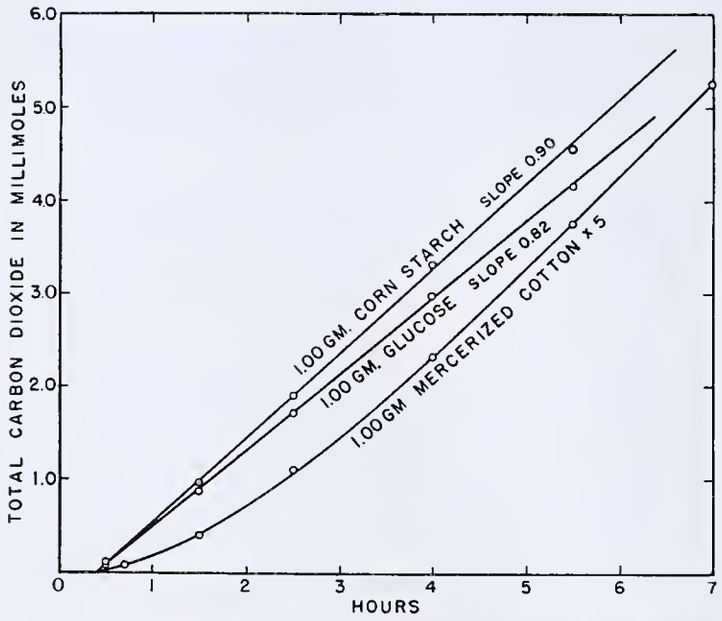
TOTAL CARBON DIOXIDE IN MILLIMOLES
HOURS
1.00 GM. CORN STARCH SLOPE 0.90
1.00 GM. GLUCOSE SLOPE 0.82
1.00 GM MERCERIZED COTTON x 5

FIGURE 6. COMPARISON OF CARBON DIOXIDE EVOLUTION RATES OF CORNSTARCH, GLUCOSE, AND MERCERIZED COTTON UNDER SIMILAR CONDITIONS

The conversion of carbon dioxide evolution rates to apparent glucose content can be made in several ways—for instance, starch, like glucose, yields an approximately linear rate curve (Figure 6). The ratio of the slopes for equal weights of the two substances is 1.10 and corresponds to 110 per cent of glucose from starch on acid hydrolysis. In the case of cellulosic materials, however, the type of curve obtained (Figure 6) requires different treatment. If the times of the observations in hours, T, are corrected for the induction period, α, then a graph on log-log paper of the observed amounts of carbon dioxide, C, from 1 gram of the material and the corrected time values, $T - \alpha$, is approximately linear.

$$C = A(T - \alpha)^B$$

where A and B are constants. The first derivative of this equation gives the slope at any time. [A similar equation has been used by Birtwell, Clibbens, and Geake (*2*) to describe the variation of copper number with time when cotton is immersed in acids.] Since a unit weight of cellulose yields 1.11 weights of glucose on complete hydrolysis, the percentage breakdown of the cellulose is given by the expression

$$\left(\frac{dC}{dT}\right)\left(\frac{100}{1.11\,G}\right) = \frac{100\,AB}{1.11\,G}(T - \alpha)^{B-1}$$

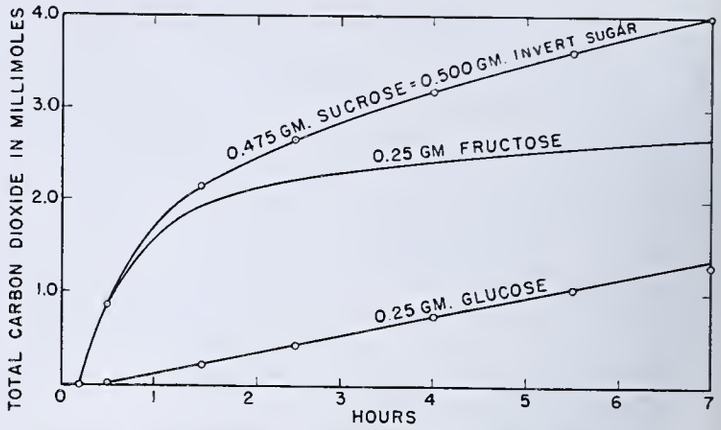

FIGURE 7. DIFFERENTIAL BEHAVIOR OF GLUCOSE AND FRUCTOSE IN ACID-CATALYST SOLUTION

in which G is the slope for 1 gram of glucose under the same conditions. Carbon dioxide lags behind the glucose content by the induction period, α; hence, the calculated percentage breakdown has occurred at $T - \alpha$.

Discussion

The method as described is applicable to glucose and to substances, such as cellulose, which yield practically pure glucose on hydrolysis. Impure materials may require correction factors—for example, there is no carbon dioxide evolved from acetic acid under these experimental conditions. It is possible, therefore, to examine cellulose acetate if a simple weight correction is made for the acetyl content.

The response of fructose, a ketose, to the catalytic oxidation is very different from that of glucose. This can be seen in Figure 7 where curves for sucrose and its components are reproduced. The curve for fructose was obtained by subtraction of the glucose component. Dissimilar amounts of carbon dioxide from different sugars in the uncatalyzed system have been reported by Norman (*6*) and by Campbell and collaborators (*3*). It appears, therefore, that the present method cannot be applied indiscriminately.

The study by this method of the breakdown rates of various cellulosic materials will be reported in a subsequent paper.

While this article was in press, a ferricyanide method for the determination of fructose in the presence of glucose was published by Becker and Englis (*1*). Their curves for glucose and fructose are strikingly similar to those in Figure 7 above.

Summary

A method is described by which quantitative, hydrolytic decomposition rates of glucose polymers, particularly cellulose, can be determined. The basis of the method is the fact that the normally slow evolution of carbon dioxide from glucose in acid solutions can be increased at will by the use of a suitable catalyst; the accelerated evolution rate is directly proportional to the free glucose in the system. Glucose set free by hydrolysis can be estimated continuously. Experimental conditions and methods of calculation are presented.

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Applicator for the Preparation of Uniform Paint Films

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A machine for spreading uniform paint films has been devised which is extremely simple in design and operation. The principle is a doctor blade having enough mass to flow automatically down an inclined plane by the pull of gravity. This device gives films which are free from the unevenness produced with hand-drawn doctor blades. Films 4 inches wide and approximately 30 inches long are prepared for exposure work, but any reasonable size desired can be made. Clear oils, varnishes, lacquers, enamels, outside house paints, thixotropic, or free-flowing flats all give uniform films by this method. With modified doctor blades, a second coat may be applied over a properly dried first coat.

THE preparation of uniform films for various types of finishes has been a rather vexing problem.

In 1922, Walker and Thompson (*8*) published the spinning-disk method for the preparation of uniform films. Modifications of this principle were the offset spinning disk and Scofield's (*5*) spinning machine, with a sloping surface. Arlt (*1*) published the spray-gun method of producing films of controlled thickness. Flowed films have been illustrated by Stoppel (*6*), and dipped films by Bruins (*3*). Films made with a roller-type machine have been described by Strieter (*7*). Doctor blade machines, the type most commonly used, are well illustrated by the Parks (*4*) Film-O-Graph, Brier and Wagner (*2*) basket blade, and Bird or Bradly blade. All the above machines and their many modifications have served a very useful purpose, but the doctor blade principle seems to be the most generally useful for handling all kinds of formulations.

With the foregoing information on the subject, an investigation was made to develop a quick and simple means of producing large enough paint films, with a sufficiently high degree of uniformity to be suitable for exposure and general physical test purposes.

Description of Machine

During the early stages of development, it was found that drawing doctor blades by hand gave poor results, especially when rather large-sized films were desired. A milling machine carriage and a hydrostatic flow arrangement were tried for producing a uniform movement of either the doctor blade or the base upon which paint was applied. Every turn of the milling machine was recorded in the film in the trials and therefore it could not be used. Hydrostatic control of the movement gave satisfactory results but was too cumbersome for general use. Finally, the pull of gravity down an inclined plane on a fair-sized doctor blade was tried, and proved to be very effective.

Once the inclined flow arrangement was decided upon it became a question of the type of doctor blade to select. The authors chose a square trough type, illustrated in Figure 1, which holds the paint and gives uniform flow with a good clean edge.

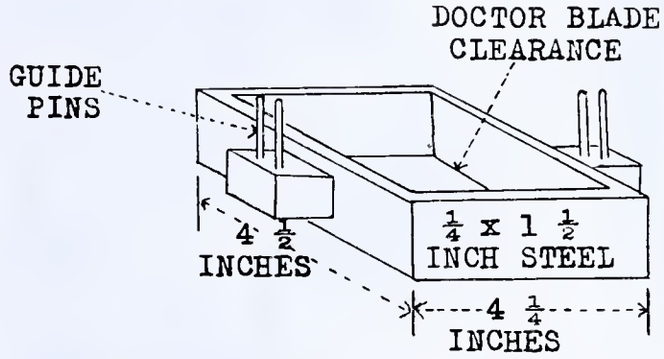

FIGURE 1. DOCTOR BLADE TROUGH FOR SPREADING PAINT

The four sides of the square trough are made of steel 4 inches long, 1.5 inches wide, and 0.25 inch thick. The four sides are pinned together and held in place by screws, and are brought to a common plane by lapping upon a flat surface. One side is cut to a definite number of thousandths of an inch below the other three sides and then reassembled. When completed, the doctor blade must be tested by preparing films on plate glass. Looking through these films with transmitted light the degree of uniformity of the paint film is readily observable, and if necessary the doctor blade is touched up with a fine stone until it gives a uniform film.

Rings could be used, as well as squares, for single-coat application work. Their overhang at the bottom would facilitate emptying and cleaning, but the rings are more troublesome to machine, produce a somewhat different flow effect, and do not give as satisfactory an edge. Furthermore, in two-coat work there would not be enough bearing surface on the rings for uniform results.

For two-coat work the doctor blade is made about 0.5 inch wider than for the first coat. Doctor blades for two-coat work are machined both front and rear to a definite number of thousandths of an inch clearance. The front side has to be machined high enough to avoid touching the first coat that was applied. A good clearance for much of the authors' work seems to be about 0.003 or 0.004 inch. The rear edge or doctor blade may then be machined to approximately 0.005-inch clearance for applying the second coat; thus it in no way touches the first coat that was applied, but holds the paint in and gives a very uniform second coat.

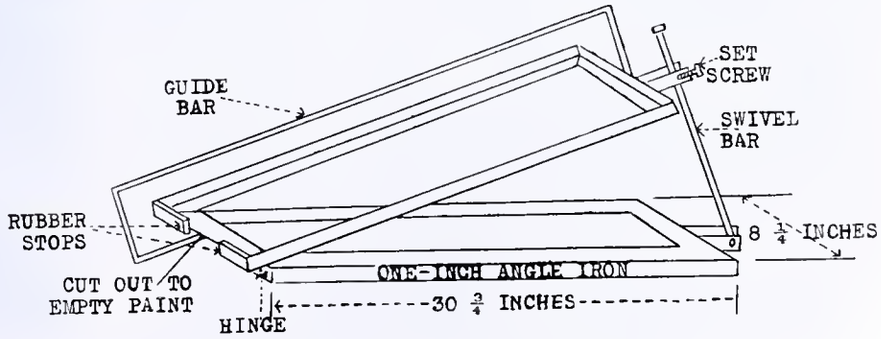

FIGURE 2. DEVICE FOR SUPPORTING PANELS

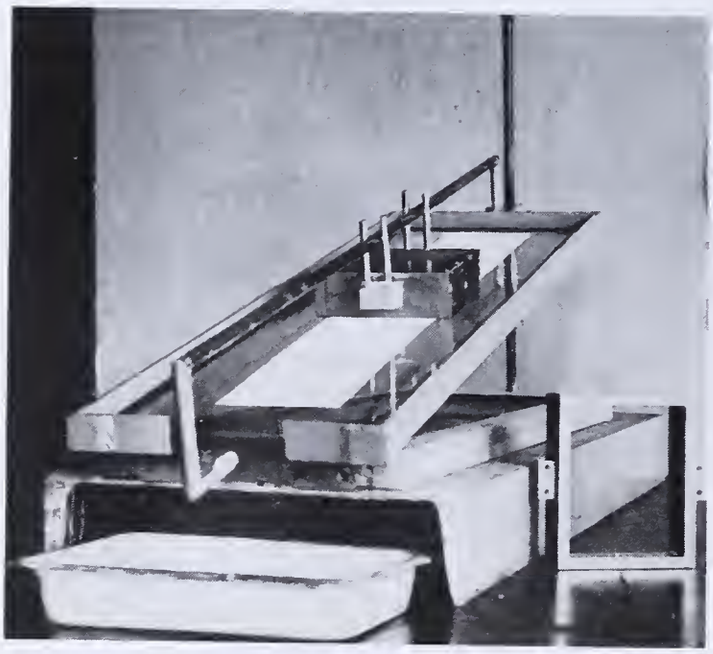

FIGURE 3. APPLICATOR FOR SPREADING UNIFORM FILM

The standard (Figure 2) for holding the stock on which the films are to be applied is made of regular 1 × 1 inch angle iron welded together at the ends. A swivel bar at one end and a hinge at the opposite end give the standard support and allow it to be tilted at any desired angle. The adjustable angle incline should be enough to eliminate the possibility of the doctor blade's sticking at any point while riding down the incline. Raising the inclined plane to a height of 9 inches gives a very satisfactory angle for plate-glass panels. In some instances, wiping the base upon which the paint is applied with thinner, or with a drop or two of the paint being used, reduces the friction and gives a smoother drop for the doctor blade. As may be noted from Figure 3, the whole machine is set up on a standard, so that a pan may be used to catch the paint at the bottom of the incline. This apparatus has been made available through R. P. Cargille, New York, N. Y.

TABLE I. FILM THICKNESS IN MILS AT POINTS MEASURED ALONG STEEL PANEL

Panel	4 Inches	8 Inches	12 Inches	16 Inches	20 Inches
1	1.2	1.3	1.4	1.6	1.6
2	1.7	1.6	1.7	2.0	1.6
3	1.8	2.2	2.2	2.0	2.1
4	1.2	1.4	1.4	1.3	1.4
5	1.8	1.8	2.1	1.8	1.8
6	1.7	2.4	1.6	1.6	1.2
7	1.4	1.4	1.3	1.5	1.2
8	1.4	1.6	1.5	1.6	1.4

Reproducibility of Apparatus

An important feature in the preparation of uniform films is the base or stock upon which paint is applied. The more uniform the surface of the stock, the more uniform the finished films. Good-grade plate glass usually has a variation of about 0.0005 inch, while cold-rolled steel in long pieces is inclined to give a little more variation, some of which is due to springing or bending the steel while handling. When the steel is properly handled better results are obtained. To indicate the reproducibility obtained with 30-inch cold-rolled steel panels eight films were spread on eight different panels, and film-thickness measurements were made every 4 inches along the center of each panel. Results given in Table I are typical values and give the expected reproducibility, considering the large area covered and the cold-rolled steel base.

On plate glass doctor blades have given excellent results, as indicated by Figure 4. These photographs were taken with transmitted light and are very uniform. Film-thickness data

TABLE II. DRY FILM THICKNESS AND RATE OF FALL OF DOCTOR BLADE

(Various types of paints for different elevations of inclined plane)

Type of Paint	Elevation of Inclined Plane from Base: 9 Inches Rate of fall	9 Inches Film thickness	11 Inches Rate of fall	11 Inches Film thickness	15 Inches Rate of fall	15 Inches Film thickness
	Seconds	*Mils*	*Seconds*	*Mils*	*Seconds*	*Mils*
Exterior finish	2.0	1.8	1.1	2.2	0.5	2.4
Wall paint	1.5	1.1	1.2	1.5	0.6	2.0
Free-flowing flat	1.4	1.6	0.8	1.8	0.6	2.0
Thixotropic flat	1.6	2.2	1.0	2.2	0.5	2.4
Enamel	4.0	1.8	3.0	2.0	1.1	2.6
Outside house paint	2.0	2.2	1.1	2.2	0.8	2.4
Pigmented lacquer	1.1	0.5	...	...	...	...
Quick-drying varnish	1.5	1.4	...	...	...	...
Raw linseed oil	1.2	1.1	...	...	...	...

for some typical surface coating materials as spread on plate glass with a doctor blade of 0.003-inch clearance are shown in Table II, which also includes the time in seconds required by the doctor blade to slide down for various angles of the incline.

It is apparent that the higher the incline, the faster the doctor blade flows down and the thicker the paint film produced. The viscosity or plasticity of the paint formulation materially affects the speed at which the doctor blade falls down the incline (Table II).

To indicate the high reproducibility of preparing films on plate glass, the data of Table III are given. These figures show that on good-grade plate glass there is a high degree of reproducibility. If panels are selected the reproducibility will be within 0.1 or 0.2 mil, as noted in Table III.

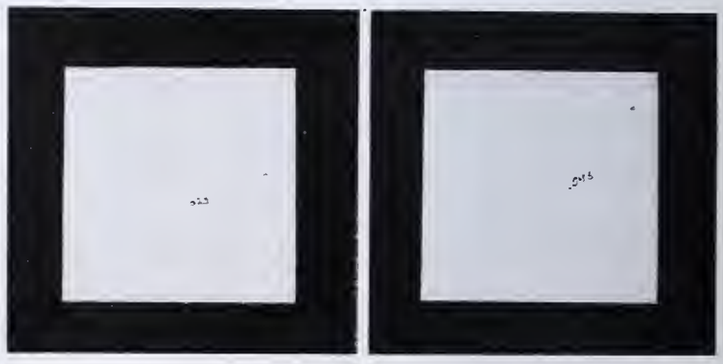

FIGURE 4. TYPICAL FILMS TAKEN WITH TRANSMITTED LIGHT
Left, flat paint. Right, outside house paint

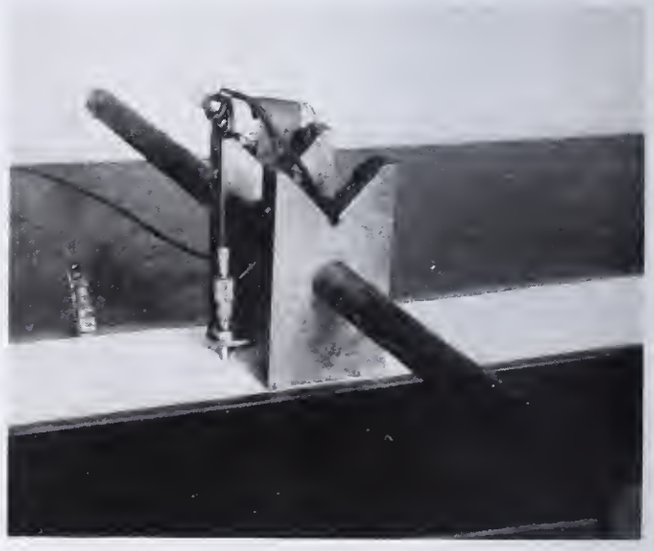

FIGURE 5. DEVICE FOR MEASURING THICKNESS

Table III. Film Thickness in Mils of Films Spread on Plate-Glass Panels

(Points measured along panels)

Paint	4 Inches	8 Inches	12 Inches	16 Inches	20 Inches
Exterior	2.1	2.1	2.1	2.2	2.2
Interior	2.0	2.0	1.9	2.0	2.0
Exterior	1.8	1.8	1.9	1.8	1.7
Exterior	2.4	2.4	2.4	2.2	2.2
Interior	2.1	2.1	2.2	2.2	2.2
Interior	2.4	2.4	2.4	2.4	2.5

The authors have doctor blades that will produce films of many different thicknesses. However, the dried film is always a bit thinner than the clearance of the doctor blade used: A film put on with a 0.003-inch blade may be anywhere from 0.5 to 2.5 mils thick, depending on the formulation of the paint and the perfection of the stock upon which the film is applied.

Measurement of the wet and dry film thickness of clear linseed oil films as well as pigmented paints containing no volatile shows that linseed oil shrinks appreciably on drying, which is a factor of importance in formulating. For example, when a paint film containing approximately 30 per cent pigment by volume shrinks 25 per cent on drying, the resultant dried film would contain approximately 40 per cent pigment by volume.

The method used for measuring the film thickness is indicated in Figure 5. The micrometer head is brazed to the block, so that the spindle may be readily brought to the surface of the paint film. For wet film thickness the spindle is screwed through the film to the metal, but for dry film thickness measurements the film is chipped or dissolved away. This makes an inexpensive but fairly accurate means of measuring film thickness. A flashlight bulb, attached for measurement of film thickness on metal, flashes on when the spindle makes contact with the metal panel. Some film thickness measurements were checked with a microscope, shims, and a regular micrometer on stripped films, so they may be considered reasonably accurate.

Acknowledgment

Many thanks are due the National Lead Company Research Laboratories for allowing the authors to publish this paper and to A. Stewart, director of research, under whose supervision the work developed.

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Presented before the Division of Paint and Varnish Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Graphite Heating Baths

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THE heating of glass flasks used in distillations, extractions, and reactions has always presented a problem. For this purpose, open flames, hot plates, or liquid baths are used at present in the laboratory, but these methods either present serious fire hazards or are inefficient.

Some years ago, the author undertook to run a series of ammonolysis reactions at high temperatures (330° C.) for extended periods (*1*). The use of oil baths with their disadvantages of decomposition and fire hazard was precluded. Metal baths were found to form mossy clumps after a while with attendant loss of material.

A glance at some of the more authoritative laboratory manuals showed no discussion of graphite as a heating medium, but the possibility of its use was mentioned by Lassar-Cohn (*2*).

A powdered graphite was tried as a heating bath material, and was found suitable for both reactions and distillations, especially where high temperatures were required—e. g., the fractional distillation of high-molecular-weight fatty compounds. Heat conductance of a properly proportioned bath was excellent; once a desired temperature was reached there was no difficulty in maintaining it. The utility of this material in ordinary laboratory operations was amply confirmed by its increased use among the author's colleagues.

The technique in the use of graphite baths is the same for ordinary laboratory distillations as for large-scale operations, where graphite has been found to be an excellent heating bath material. An iron container is selected of such a size as to be only slightly larger than the flask to be heated, and enough graphite is placed in the container to form a floor for the flask, a 0.25-inch layer being ample. The flask is placed on the layer of graphite and held in position by the conventional system of clamps. Enough graphite is added to surround the flask, after which the material is tamped to form a tight packing. When it is desired to note the bath temperature, a thermometer is suspended near the wall of the flask, with the bulb at the height of the lower third of the flask. The bath is heated with either a Bunsen or Méker burner, depending upon the temperature desired.

Variation in size of the graphite particles produced no significant difference in efficiency of heating. The flake size (Dixon No. 1, large flake) seems best suited, for it does not soot and is cleaner to handle.

The cost of graphite and its permanent quality encourage its use for both ordinary and large-scale laboratory operations.

Acknowledgment

The author wishes to thank E. S. Glauch of The Joseph Dixon Crucible Co., Jersey City, N. J., for supplying liberal samples of various grades of graphite for experimental purposes.

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Modified Photoelectric Photometer

For Colorimetric Determinations in Water and Sewage Laboratories

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Sanitary District, Decatur, Ill.

A vertical-type photometer, utilizing 1 or 2 photronic-type cells and low-form 100-ml. Nessler tubes for absorption cells, is described. The single-cell unit can be built for $26 and the balanced-cell unit for $60, including four light filters with light transmissions of 390 to 510, 430 to 540, 490 to 590, and greater than 500 millimicrons. Other light filters are described for special work, the filters costing less than 50 cents each.

The two units give practically the same standardization curves. The single-cell unit is tedious in operation, because of fluctuations in light intensity. The balanced circuit is free from such fluctuations and costs less than voltage regulators. The low-form 100-ml. Nessler tubes were ruled with inside depths of 25, 50, 75, 100, and 150 mm. The optical properties of these tubes (Exax) have been similar, about 50 per cent giving identical readings, and thus the tubes can be used interchangeably.

Colorimetric pH can be determined with greater accuracy than by visual methods, using only 0.25 ml. of indicator in 25 to 100 ml. of sample. Turbidity is easily determined and compensated for in the same sample.

After standardizing the instrument, nitrates, nitrites, ammonia, residual chlorine, iron, manganese, and even iodine in the D. O. and B. O. D. determination may be determined from the standardization curves, thus doing away with color standards or permanent standards which often do not have the same absorption spectrum as the unknown color.

DURING 1939 Müller and many others (*1*, *6*, *7*) published excellent summaries, including complete bibliographies, on photoelectric methods and circuits for use in analytical chemistry.

Many laboratory supply houses offer excellent photoelectric photometers, costing between $150 and $300 and even $500 or more. These instruments are of a horizontal-beam type and only the more expensive ones will accommodate absorption cells 100 to 150 mm. long. These long cells cost between $16 and $20 each and for routine work (particularly with sewage containing grease) are unhandy for filling, refilling, and cleaning. Many of the colorimetric determinations in water and sewage work require deep cells of the Nessler-tube type, because of the very small quantities being determined. The high cost of these photometers and cells precludes their use in any but the large water and sewage laboratories. For this reason the authors have developed "vertical"-beam photoelectric photometers using one unbalanced and two balanced circuits, and a technique for using modified short-form 100-ml. Nessler tubes as absorption cells. The cost of material for these instruments is $26 and $60, respectively, including light filters, and the cost of the Nessler tubes is about $1 each.

Photoelectric Circuits

Three photoelectric circuits have been built. *A* is the unbalanced simple type described by Hurwitz (*3*) which was built for $26, *B* is the balanced series photronic cell circuit described by Brice (*2*), and *C* is the balanced parallel photronic cell circuit described by Winthrop, Shrewsbury, and Kraybill (*9*).

Circuits *B* and *C* are twice as expensive to build but have the distinct advantage of being independent of changes in light intensity due to voltage fluctuations when laboratory heating units or automatic pumps go on or off the power circuit. However, with patience, excellent results may be obtained with *A* by always checking the blank both before and after each unknown reading and calculating the per cent transmissions, rather than trying to keep the machine set at 100 for the blank and reading the transmissions direct from the scale. Photographs of *A* and *B* and the wiring diagrams of *A*, *B*, and *C* are shown in Figure 1.

The light source used in these photometers is a 150-watt Mazda projector spot bulb which concentrates and straightens out the light beam sufficiently, so that lenses have been found unnecessary. The galvanometer is sensitive to 1 micro-ampere per mm. scale division. A greater sensitivity would make the setting of the apparatus too delicate for practical work. As Brice pointed out, the resistance in the galvanometer and the circuit should be very low compared to the internal resistance in the photocells. Therefore the resistance of this galvanometer is 22 ohms and the damping resistance is 15 ohms. The slide-wire rheostat is vernier-controlled and has an approximate resistance of 28 ohms. (This does not have to be exact, so long as the operating resistance is above the damping resistance of the galvanometer.) This rheostat was easy to scale into 100 per cent divisions and the

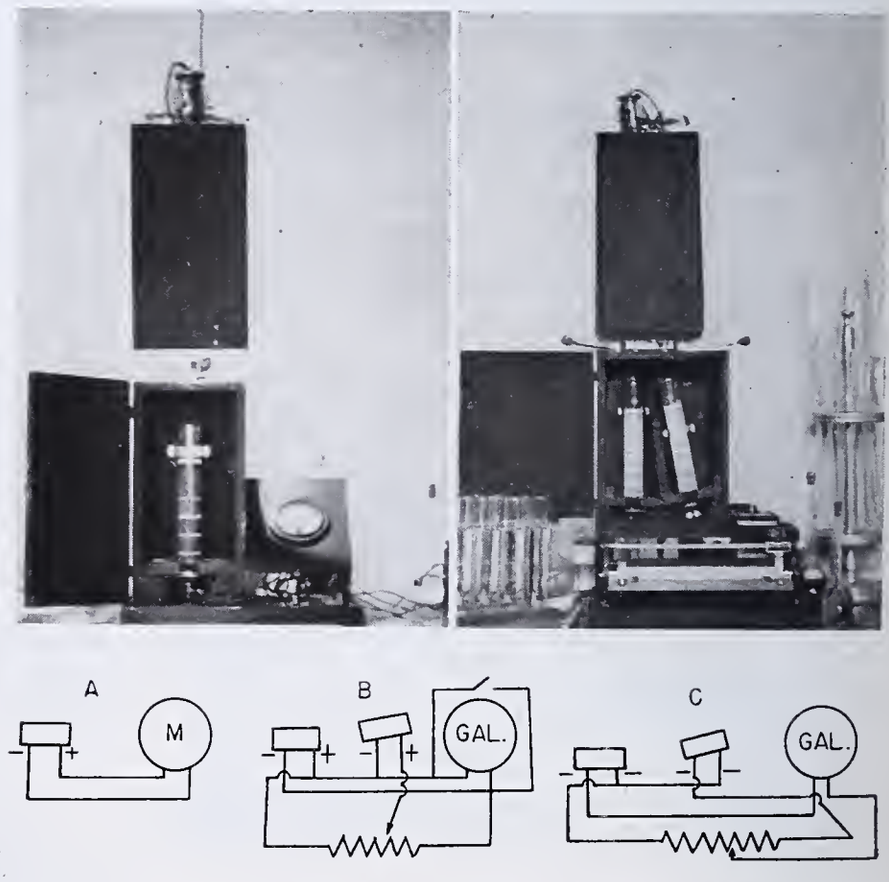

FIGURE 1. PHOTOMETERS AND WIRING DIAGRAMS

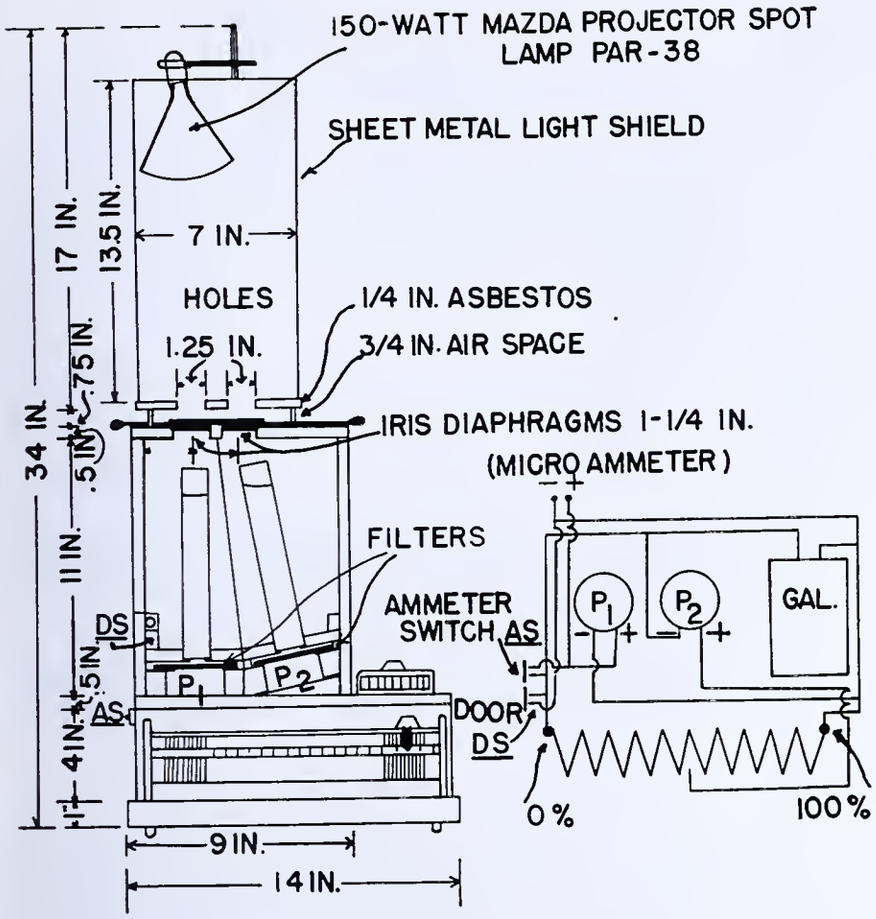

FIGURE 2. WIRING DIAGRAM

scale has been found very accurate by calibration against Leeds & Northrup resistance boxes. The photronic cells used are No. 594, Type 1, disk 69,902, which are sold particularly to match with microammeter 301. These cells are about three times as sensitive as those usually furnished when No. 594, Type 1, cells are ordered without specifying the disk number and not purchased with the microammeter 301. The specifications and cost of materials after discount are presented below.

Circuit *A*

1 Weston photronic cell No. 594, Type 1, D69,902	$ 6.30[a]
1 Weston microammeter No. 301, 360 ohms resistance	9.41
1 iris diaphragm 1.25-inch diameter, Chicago Apparatus Co.	5.28
1 150-watt Mazda projector spot bulb PAR-38	1.05
4 Wratten gelatin filters, Eastman Kodak Co., Nos. 12, 45H, 47A, and 61N, at 40 cents	1.60
4 each, Eastman thin cover glasses for lantern slides, 3.25 × 4 and 2 × 2 inches	0.31
Miscellaneous lumber, screws, switches, etc.	2.05
Total	$26.00

Circuit *B* (preferred)

2 Weston photronic cells No. 594, Type 1, D69,902	$18.90[a]
1 G-M Laboratories, Inc., No. 2561-D galvanometer (without case)	16.00
1 G-M Laboratories, Inc., No. L-28-4.0 vernier action rheostat	6.38
2 iris diaphragms, 1.25-inch diameter opening	10.54
1 150-watt Mazda projector spot bulb PAR-38	1.05
8 Wratten filters, Eastman Kodak Co., 2 each of Nos. 12, 45H, 47A, and 61N	3.20
8 each, Eastman thin cover glasses for lantern slides, 3.25 × 4 and 2 × 2 inches	0.62
1 Philco refrigerator door switch, as galvanometer shunt	0.77
Miscellaneous lumber, screws, switches, etc.	3.00
Total	$60.46

[a] These cells when purchased with Weston microammeter No. 301 list at $10 less 37%; without microammeter, at $15 less 37% discount.

CIRCUIT *C*. The same materials are used as listed above for *B* except for one additional 100-ohm fixed resistance ($1.25) and a rheostat of 100-ohm resistance which should be balanced against the 100-ohm fixed resistance. In circuit *B* the rheostat is not balanced against another resistance and may be 20 to 30 ohms or less, or just so it does not damp the galvanometer. For these reasons *B* is preferred to *C*.

Identical results have been obtained with all three instruments, but because of the inconvenience of voltage variations with *A*, and the more complicated resistance balance in *C*, *B* is recommended for the operator wishing to build his own apparatus. Details of construction are given in Figure 2.

The light source is attached to a 0.63-cm. (0.25-inch) pipe (0.94-cm., 0.375-inch, outside diameter) which is firmly attached to the base and frame of the apparatus. The bulb socket is held in a buret clamp and clamp holder, thus affording easy adjustment of the light bulb so that it focuses equally on both photo cells. Switch *DS* is a Philco refrigerator door switch which shunts the galvanometer whenever the door is opened and absorption cells are being changed. Switch *AS* and the plus and minus outlets at the rear of the apparatus are not necessary to circuit *B*. With *AS* closed the apparatus functions as the Brice balanced cell circuit but with *AS* open and microammeter attached to the plus and minus outlets as shown, photocell P_1 acts alone so circuit *A*, thus affording an easy way to switch from one circuit to the other. In building *B AS* can be eliminated and the circuit closed at this point.

FILTERS. The Wratten gelatin light filters made by the Eastman Kodak Co. have proved satisfactory and most economical. Although they are not so permanent as glass filters, they are so cheap that they can be renewed frequently. They were placed between two Eastman thin glass lantern slides 8.1 × 10 and 5 × 5 cm. (3.25 × 4 and 2 × 2 inches), and sealed from air and moisture with Scotch cellulose tape used for mounting color films. Each mounted filter cost about 48 cents complete. These slip into the apparatus just under the Nessler-tube absorption cell

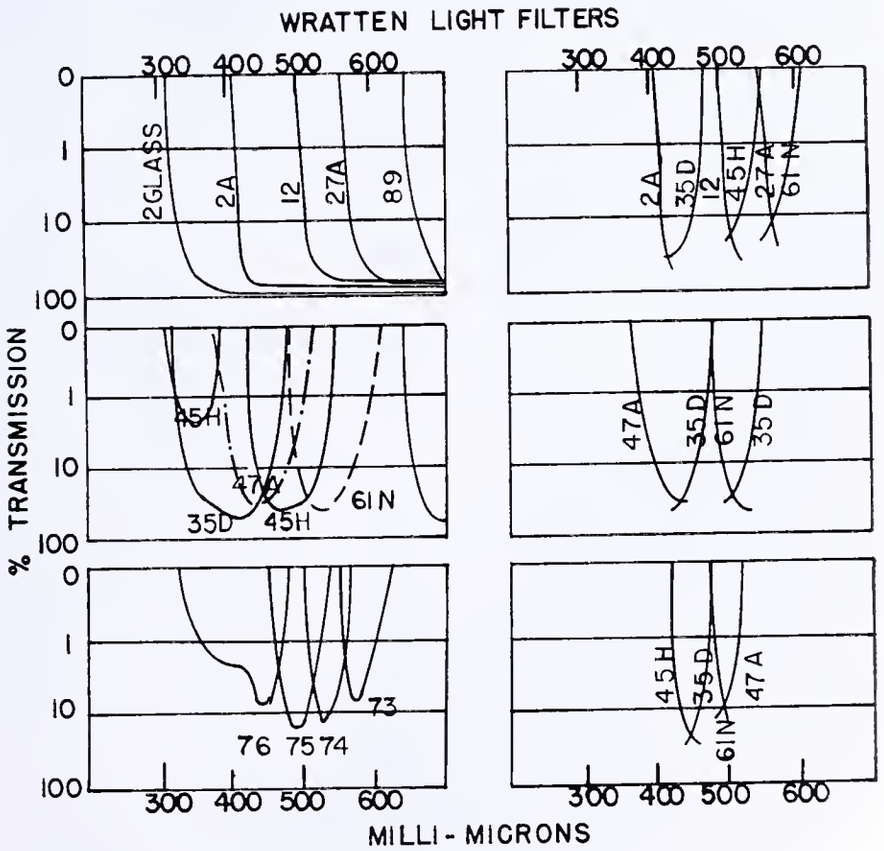

FIGURE 3. TRANSMISSIONS OF WRATTEN FILTERS

TABLE I. WAVE TRANSMISSIONS OF WRATTEN LIGHT FILTERS

(Data from Eastman Kodak Co.)

Filter No.	Wave Transmission *Millimicrons*
2A	410+
12	500
27A	560
89	650
35D	310–480 and 650+
47A	390–510
45H	320–390, 430–540
61N	490–590
76	320–470
75	460–520
74	510–560
73	560–600
Filter Combinations	
2A and 35D	410–480
47A and 35D	390–480
45H and 35D	430–480
61N and 47A	490–510
61N and 45H	490–530
12 and 45H	500–530
12 and 75	500–520
27A and 61N	560–590

and above the photronic cell and are therefore not easily harmed by heat from the light bulb. These filters have been used for 2 years without deterioration. Although for water and sewage work four filters have served all purposes (Nos. 12, 45H, 47A, and 61N) it is convenient to have a rather complete set of filters giving narrower transmission bands. The transmissions of 12 Wratten filters and combinations of these filters are given in Table I and Figure 3.

ABSORPTION CELLS. Low-form 100-ml. Exax Nessler tubes were marked for the authors by the Kimble Glass Co. for inside depths of 25, 50, 75, 100, and 150 mm. at a cost of less than $1 each, but without the 100-ml. volume calibration. In the future the authors would purchase tubes with both the depth markings and the 100-ml.volume calibration, so that the same cell may be used for both measuring out the volume and then removing the amount necessary to leave a depth of 150 mm. for absorption cell testing. Between 40 and 50 per cent of the tubes furnished give identical transmissions when filled with distilled water, and may therefore be used interchangeably. This greatly facilitates routine work. However, the cells that differ from the average by a few scale divisions on the galvanometer may also be used, provided the blank and unknown absorptions are made in the same cell.

SCALE. The per cent of light transmitted by the unknown is determined by balancing the current produced by cell P_1 with that from P_2, the latter being adjusted by introducing resistance in the P_2 part of the circuit. The scale for the rheostat is made by dividing the length of the resistance coil (which was 254 mm. on the G-M rheostat used) into 100 equal parts representing per cent current from P_2, the 100 per cent being obtained when there is no resistance in the P_2 side of the circuit. The scale is drawn on a strip of frosted glass and is mounted on the rheostat, so that the 100 per cent is indicated by the first deflection of the galvanometer as the vernier adjustment is moved to the left. Likewise the zero on the scale may be checked by blocking all the light from P_1 and adjusting the lower end accordingly.

ZERO BLANK SETTINGS. All three circuits are so designed that they read directly in per cent transmission, the apparatus being adjusted to read 100 per cent for the zero blank. This blank should contain none of the unknown substance, and should be treated in the same way and with the same reagents as the unknown sample. With circuit A the blank setting is 100 microamperes and with B and C the scale setting is 100. These adjustments are made with the single iris diaphragm when using A, and by adjusting both diaphragms so that the galvanometer reads zero (center) when using B or C circuits. With the latter circuits identical results are obtained with light intensities which produce 50 to 150 or 200 microamperes current in the photronic cells. However, the larger currents make the zero-blank settings so tedious that most tests are made at the 50-microampere level. The authors have roughly calibrated each filter so that they know the diaphragm opening which gives about 50 microamperes with distilled water blanks 150 mm. deep. With the apparatus, as constructed, the following diaphragm openings give 50-milliampere current from the cells:

Wratten Filter No.	Diameter Opening *Mm.*	Per Cent of Area
12	3.5	1.25
45H	12.	14.05
47A	15.	22.
61N	10.	9.55
Wide open	32.	100.

The adjusting levers of the iris diaphragms should have a friction brake, so that they are not too easily moved for adjustment and so that after adjustment they will not change when the door is opened or closed.

The water level in the Nessler tube above P_2 is unimportant, since this tube is used only as a light condenser for P_2, but the liquid levels in the blank and unknown must be carefully measured to the desired depth which was used in the calibration of the instrument for the particular determination.

Procedure

With instrument A the proper filter is inserted between the Nessler tube and the photronic cell, the tube containing the zero blank is inserted, and the iris diaphragm is adjusted until the microammeter reads 100 (the meter specified reads 200, so that this reading is in the middle of the scale). The unknown tube is now inserted in place of the blank and the reading of the meter is noted. Immediately the blank is reinserted and if it still reads 100 the per cent transmission of the unknown is indicated directly. If the blank reading is more or less than 100 the unknown must again be inserted and the process repeated until it is certain that the blank and unknown readings are taken on constant voltage. Then the per cent transmission is calculated from the final blank and unknown readings.

PROCEDURE WHEN USING B AND C. Put proper "filter pair" in place, insert tubes of distilled water above P_1 and P_2, adjust iris diaphragms approximately for filters being used, and turn on light, allowing 15 minutes for equilibrium to be reached. Replace cell above P_1 with zero blank of exact depth and adjust iris diaphragm on the left until the galvanometer reads zero. If the

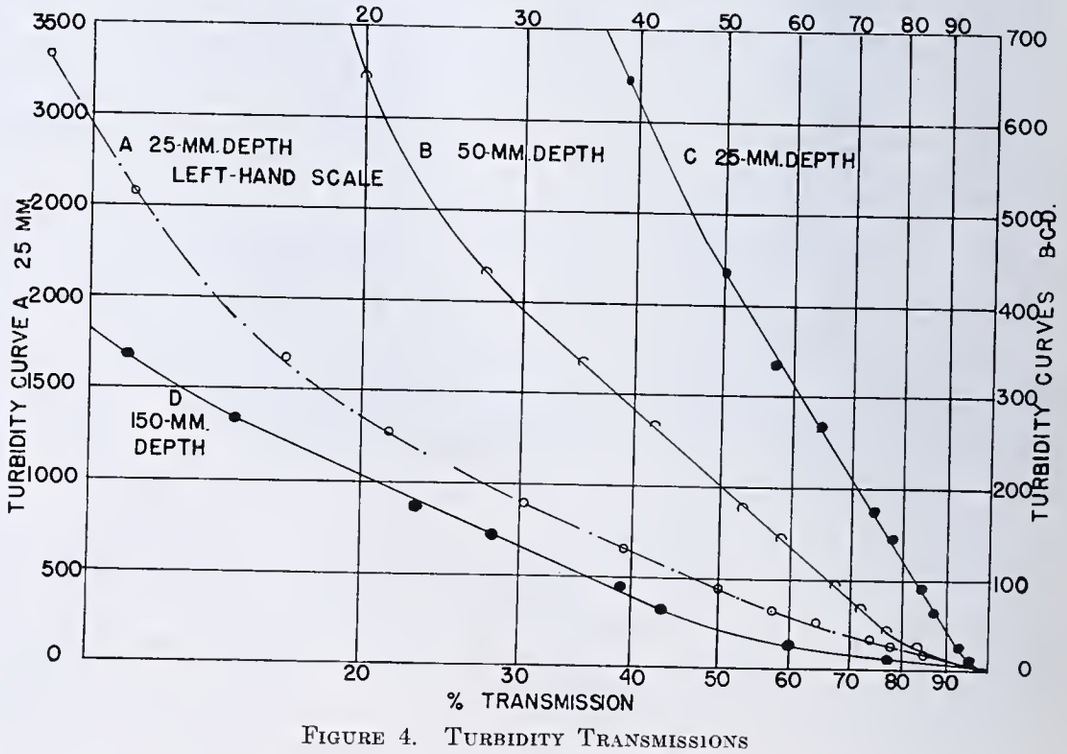

FIGURE 4. TURBIDITY TRANSMISSIONS

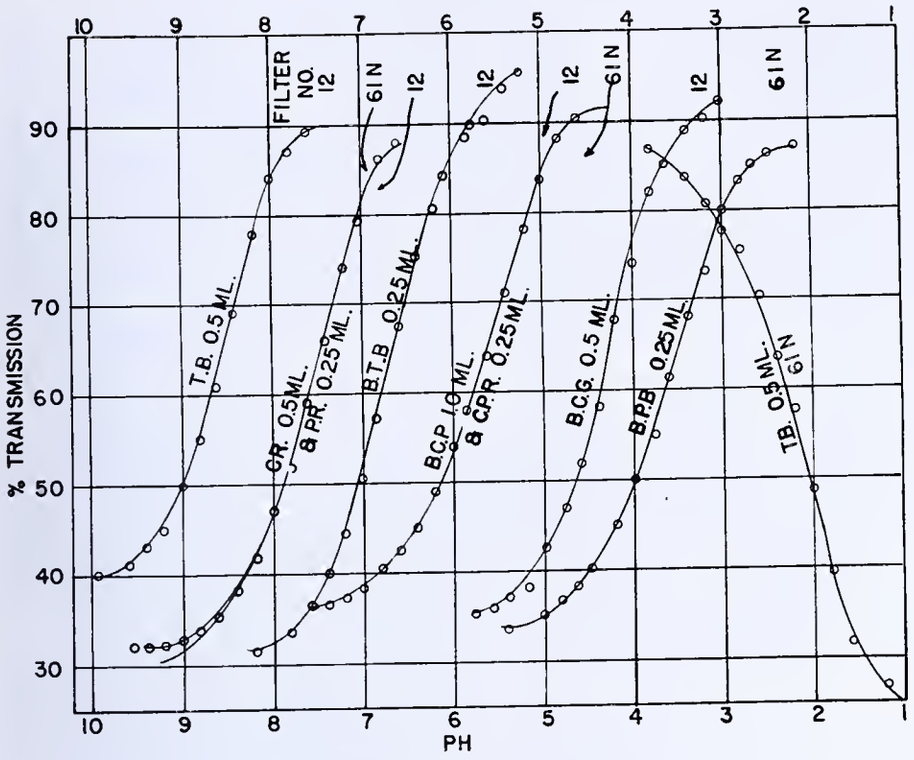

FIGURE 5. pH INDICATOR TRANSMISSIONS

galvanometer is too sensitive cut the light to the cells by reducing the opening in both diaphragms a little. Having obtained exact balance, remove the blank and insert the unknown of same depth. If the color is deep, move the rheostat to 30 or 50 per cent before closing the door to protect the galvanometer. With the vernier control, adjust the rheostat to exact balance and read the transmission of the sample from the scale. Determine the amounts of unknown from calibration curves.

CALIBRATION. The instruments are calibrated for each determination by using color standards prepared according to Standard Methods of Water Analysis (*4*), except that in general 100 ml. of the color standards are prepared. After the proper time interval for color formation the standards are measured into the absorption tubes, usually to the 150-mm. depth line, and the transmissions determined. The transmission curves obtained when the results are plotted on semi-log paper replace all color standards.

Determinations

TURBIDITIES. The determination of raw water and sewage turbidities is very easily accomplished with the photometer, using a 25-mm. depth for turbidities of 20 to 3500 p. p. m. and a 50- or 150-mm. depth for the lower ranges from 10 to 400 p. p. m. No attempt has been made to develop a technique for effluents below 10 p. p. m. The curves shown in Figure 4 represent calibration curves made with bentonite clay suspensions, the turbidities of which were determined with a Jackson turbidimeter. These curves were made using a No. 12 filter. Experiments have shown that unfiltered light and filters 2A, 12, and 27A give the same turbidity curves, while the narrower band filters give 1 to 2 per cent greater transmissions. Because the authors' routine provided for subsequent determination of pH with bromothymol blue, and because for this indicator they use filter 12, they used No. 12 for their turbidity calibration. The samples should be introduced into the Nessler tubes with a pipet, so that drops are not splashed and do not remain on the side walls of the tube.

HYDROGEN-ION CONCENTRATION. The Clark sulfophthalein indicators give beautiful calibration curves using 25-mm. (or greater) depths in the absorption tubes. Only two filters were found necessary for the entire pH range from 1 to 10; filter 12 was used for thymol blue (alkaline), bromothymol blue, bromocresol purple, and bromocresol green; filter 61N was used for cresol red, phenol red, chlorophenol red, bromophenol blue, and thymol blue (acid). The curves shown in Figure 5 are plotted on standard cross-section paper from data obtained with carefully prepared Clark buffer solutions, using the exact quantity of indicator and the filter indicated at the right end of each curve. The depth was 25 mm. of buffer, to which was added the amount of indicator shown. The exact depth of solution may vary, so long as the blank has the same depth.

In determining pH the absorption is due to the color and the concentration of the indicator, so that care must be taken to add the exact amount of indicator used in the calibration. One-milliliter pipets graduated in 0.01 ml. were satisfactory in this respect. The concentrations of freshly prepared indicator solutions must be checked because they often vary slightly in intensity. This is easily done by choosing a buffer at about mid-point on the curve and finding how much indicator is necessary to produce the transmission given by the calibration curve. For example the new indicator solution may require 0.27 ml. instead of 0.25 ml. to produce the transmission required, particularly when a new supply of indicator salt has been used.

Since it is easy to read the transmissions to within ±0.5 per cent, the calibration curves on cross-section paper may easily be read to the second decimal place. However to justify such accuracy one must be sure that the calibration buffers are equally accurate. In general, accuracy greater than 0.1 pH is not justified.

COMPENSATION FOR TURBIDITY AND COLOR. The photometer technique easily compensates for turbidity and color in the water or sewage. For example, after determining the transmission on a sample 25 mm. deep for turbidity and/or color, the iris diaphragm is opened, with the rheostat again set at 100, until the zero galvanometer reading is again obtained, then the indicator is added with the pipet extending almost to the liquid surface, and the transmission is again determined. [The authors have not had the occasion nor facilities to study natural color in water. By the proper choice of filters, turbidity and color might easily be separated and determined (*5*, *8*).] With the aid of the calibration curves the pH is easily determined. With unsettled sewage turbidities greater than 300 p. p. m. were found to absorb the indicator and give false readings, but this difficulty was not encountered when the sewage was settled to remove the coarser particles.

SLIGHTLY BUFFERED WATERS. In order to determine the pH of slightly buffered waters, isohydric indicators must be used or the volume of water must be large compared to the amount of indicator used. The technique using a 25-mm. depth has been satisfactory on the buffered sewage used in the authors' routine; however, 150-mm. depths would do equally well, thus using only about 0.25 ml. of indicator in slightly less than 100 ml. of water. In fact, for water analysis, 150-mm. depths would probably be the best for turbidity and pH determination. Some of the curves will be the same for 150 mm. as for 25 mm., but most of them will be changed

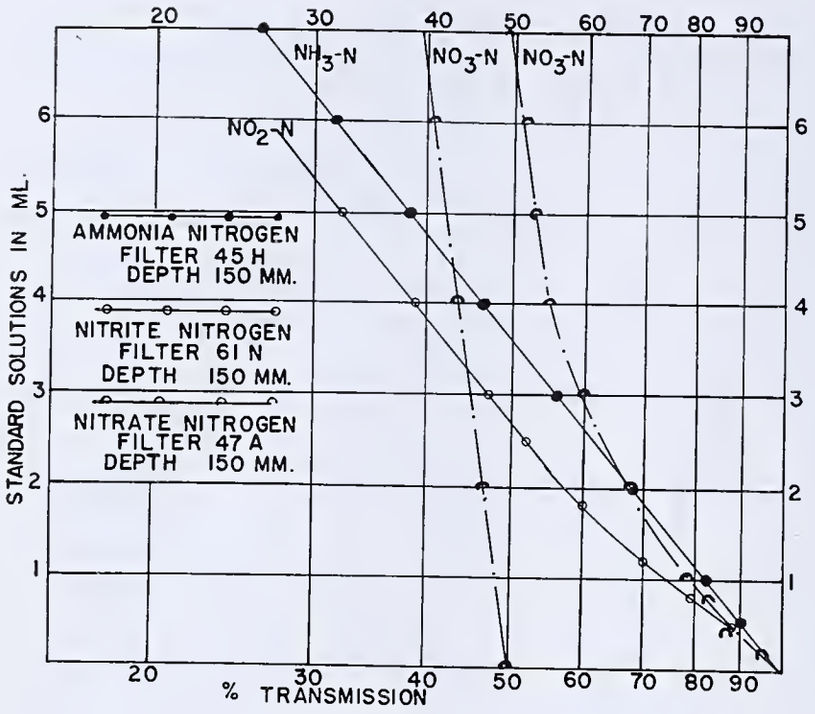

FIGURE 6. AMMONIA, NITRITE, AND NITRATE NITROGEN

about 2 per cent. In pH work the exact volume of water is not important, but the amount of indicator in the tube cell must be accurately measured.

pH OF THICK SLUDGES. It is often difficult, with sewage sludges, to obtain a sufficiently clear supernatant liquor for colorimetric work, and the dilution procedure (*4*) is often unsatisfactory because the buffer value of the sludge will not stand dilution. The authors have found that a clear sample for colorimetric pH may be easily and accurately obtained by diffusion through dialyzer parchment paper or cellophane.

Diffusion cells were made by cutting off the bottoms of 120-ml. (4-ounce) glass bottles and covering the opening with parchment paper by means of a rubber band. The inside of the cell was then filled with aerated distilled water (pH 6.9 to 7.0) and suspended in a large beaker of sludge, the two liquid levels being about equal. Repeated tests proved that within 15 minutes the distilled water had reached equilibrium with the sludge, and a perfectly clear solution was available on which to run colorimetric pH determinations. Dialyzer tubing may be used instead of the cell, although the cell is more easily handled than the tubing.

This diffusion method is the most satisfactory method the authors have found for Decatur sludges with pH from 5.0 to 7.0 and is recommended as superior to dilution, centrifuging, settling, or filtering.

Ammonia, Nitrite, and Nitrate Nitrogens

The determination of ammonia, nitrite, and nitrate nitrogens has been accomplished with the photometer with equal and usually greater accuracy than by visual comparisons of color (*4*) using 50-ml. tall-form Nessler tubes. Slight turbidities interfere with the transmissions of these colors, but by coagulating a 200-ml. sample of the sewage, effluent, or river water with copper sulfate, ferric chloride, ferrous sulfate, or alum, and sodium hydroxide those elements causing turbidity and off colors are precipitated. The clear supernatant liquor without filtration may often be used for direct nesslerization of ammonia and for the nitrite and nitrate determinations (*4*). However, a fine floc which does not settle easily should be filtered. Experience with the photoelectric cell has made the authors lose confidence in visually estimating color intensities which are masked by slight turbidities or are slightly off color. When necessary, turbidities may be compensated for by determining the absorption due to the turbidity when using a filter which transmits light waves that are not absorbed by the colored solution and then correcting the transmission obtained with the filter used for the unknown substance. For example, the nitrite color gives very good absorption with filter 61N, but shows no absorption with filter 35D. Therefore the absorption due to turbidity may be determined with 35D and that amount added to the transmission when using filter 61N. This correction has been found valid for slight turbidities which occur in routine work. However, with proper coagulation the need for this correction may be eliminated.

In Figure 6 the calibration curves with the respective filters are given, the transmissions being plotted against the milliliters of standard solutions used to obtain the colors. In this way all three curves may be plotted on the same scale, although in each case the number of milligrams of nitrogen per milliliter of standard is different.

PROCEDURE. A sample of sewage, effluent, or turbid water is poured into a 250-ml. bottle to which is added 1 ml. of the coagulant: 10 per cent copper sulfate, 3.6 per cent ferric chloride, 3.6 per cent ferrous sulfate, or 3.6 per cent alum. The sample is mixed by inverting 3 or 4 times, 1 ml. of 30 per cent sodium hydroxide solution is added, and the sample is mixed by inversion and allowed to settle.

Ammonia Nitrogen. One to 10 ml. of the supernatant liquor are diluted to 100 ml. with ammonia-free water, and nesslerized with 2 ml. of Nessler reagent. Color formation is rapid in concentrations greater than 0.01 mg. of nitrogen in 100 ml. and may be read in the photometer after 30 minutes, but with concentrations of 0.001 to 0.005 mg. in 100 ml., 45 to 60 minutes are required for complete color formation. The 10-minute period for color formation recommended in "Standard Methods" (*4*) may be satisfactory for visual comparison, but the photronic cell is so much more sensitive that in most determinations a longer period for color formation has been found necessary, especially with the lower concentrations. After complete color formation the solution is poured into the transmission tube to the depth of 150 mm. and the transmission determined (having already carefully set the photometer at 100 with a zero blank) with light filter

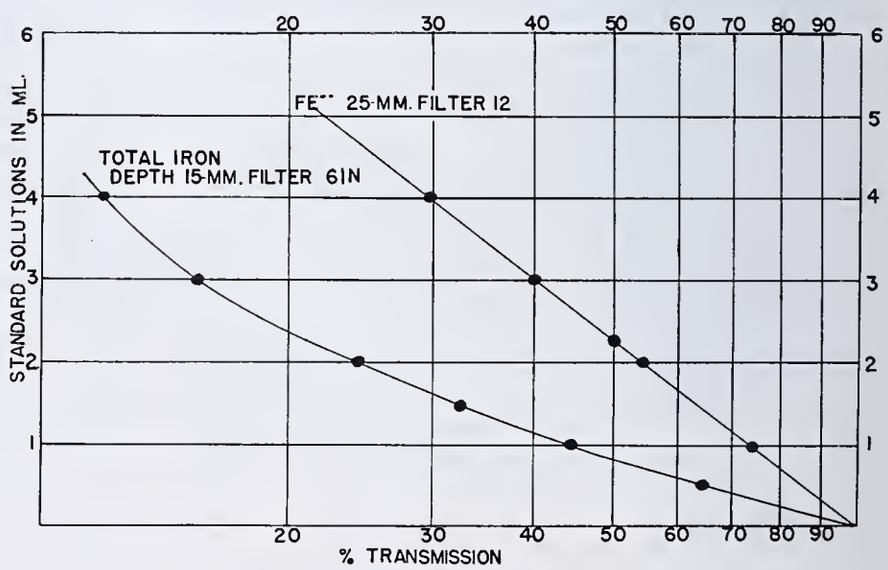

FIGURE 7. FERROUS AND TOTAL IRON

45H. From the calibration curve, the number of milliliters of standard ammonia solution corresponding to the per cent transmission is obtained and the p. p. m. of ammonia nitrogen calculated as follows, when V equals milliliters of sample used for test:

$$\text{P. p. m. of } NH_3 - N = \frac{1000}{V} \times 0.01 \times \text{ml. of standard solution} = \frac{10}{V} \times \text{ml. of standard solution}$$

Nitrite Nitrogen. Similarly 1 to 10 ml. of the clarified supernatant liquor are diluted to 100 ml. with nitrite-free water to which 2 ml. each of the two reagents are added. The transmissions are determined on 150-mm. depths, using filter 61N after setting the photometer with a zero blank made with nitrite-free water and the reagents. With the aid of the calibration curve the quantities may be calculated. A period of 30 minutes is allowed for color formation.

Nitrate Nitrogen. The nitrate nitrogen may be determined by either the reduction or phenoldisulfonic acid method. By the latter method 10 ml. or less of the supernatant liquor are treated according to "Standard Methods" (4) and the resulting color is diluted to 100 ml. The transmission of the colored solution is determined using a 150-mm. depth and filter 47A. For accuracy quantities of the original supernatant liquor should be taken, so that readings not greater than 4 ml. of standard solution—i. e., transmissions of less than 60 per cent—are obtained.

The above photometric methods have checked repeatedly against the reduction method, which the authors have always used for the routine determination of nitrite plus nitrate nitrogen in the final trickling filter effluent—i. e., the sum of the photometric nitrite and nitrate determinations checked the reduction method which was run in the standard visual way.

Ferrous and Total Iron. These have been determined by the technique described above using the procedures of "Standard Methods" (4) to produce the color. With ferrous iron the absorptions are so high that the transmissions are determined on 25-mm. depths and with filter 12, but the transmission of the ferric iron color is made at the usual 150-mm. depth and with filter 61N. The calibration curve is shown in Figure 7.

Dissolved Oxygen. It was a happy surprise to find that dissolved oxygen could be determined almost as accurately in the photometer as by titration with standard 0.025 N sodium thiosulfate, by measuring the transmission of the iodine color at 150-mm. depths with filter 61N. In biochemical oxygen demand work no correction for turbidity has been found necessary if the bottles are allowed to settle 30 minutes after acidification and the clear iodine solution is pipetted from the upper portion of the bottle. However, if there is a foreign turbidity, the absorption due to the turbidity is easily determined by adding a few drops (avoiding an excess) of 10 per cent sodium thiosulfate to destroy the iodine color and then determining the absorption of the colorless but turbid solution. The transmission of the turbid iodine sample is then corrected for the absorption due to the turbidity.

The calibration curve, Figure 8, was made by preparing a series of dissolved oxygen samples using well water, determining the transmissions, and titrating 200-ml. samples with 0.025 N sodium thiosulfate. Routine determinations of the B. O. D. dissolved oxygens have also been plotted. This is a double curve, the left-hand portion being read from the scale on the left of the figure.

Residual Chlorine. Also on Figure 8 are two calibration curves for residual chlorine, using *o*-tolidine and standard chlorine solutions which were made from BK hypochlorite so that 1 ml. contained 0.1 mg. of chlorine. These two curves illustrate the advantage of two different light filters. With No. 47A, residual chlorine may be very accurately determined between 0.1 to 0.5 p. p. m. but above 0.5 the curve rapidly becomes of no value. If one is interested in residuals from 1 to 5 p. p. m. filter 61N can be used to advantage.

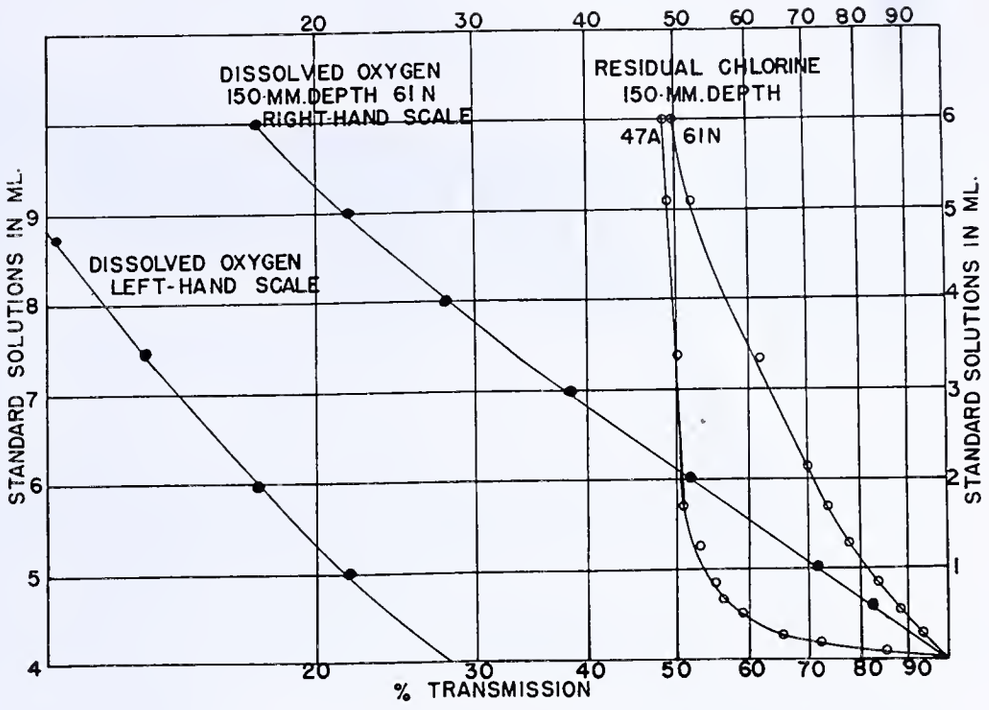

Figure 8. Residual Chlorine and Dissolved Oxygen

For routine control of water and sewage chlorination, both day and night the photometer offers a solution.

Kinks in Operation

Operation of the photometer is easy and accurate.

The light bulb should be directed so that with equal openings in the two diaphragms and equal depths of water in both Nessler tubes, the galvanometer reads approximately zero.

The photometer must have reached equilibrium before the zero setting. At least 15 minutes are allowed for the photocells to come to equilibrium.

For accurate work care must be taken that the zero blank is representative of the samples and the calibration curve. If the photometer is not properly set at 100 with a good zero blank the determinations are worthless.

During the winter months, when the laboratory had a zero humidity, no trouble was experienced from finger prints on the Nessler transmission tubes, but with warm, humid summer weather such fingerprints will throw the results completely off. Taking a tip from the safe blower, the tubes are first wiped off with alcohol and then handled with a pair of old silk gloves. This has eliminated all trouble with finger prints. The insides of the tubes are cleaned with cleaning solution and occasionally with strong lye. It pays to have the tubes scrupulously clean. If the photometer is in continuous use for 2 or 3 hours vapor rising from the surface of the tube above cell P_2 may collect on the glass above the water and cause a drift in the galvanometer. The tube is taken out and refilled with cool water as full as possible to reduce the area on which the vapor will collect.

If others than the parts specified are to be used in the construction, it is important that the resistances in the cells, the rheostat, the galvanometer coil, and the galvanometer damping resistance should be properly correlated.

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Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Determination of Exchangeable Bases in Soils

Rapid Micromethods

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Detailed time-saving procedures are described for the determination of the exchangeable bases in soils, especially suitable for routine soil testing where a high degree of accuracy is usually not required. The bases are extracted with 1 *N* ammonium acetate solution. The exchange capacity is determined by distillation of the ammonia adsorbed by the soil. Calcium is determined volumetrically. Colorimetric methods are employed in the determination of magnesium, potassium, and manganese utilizing a photoelectric colorimeter. Data tabulated show typical recoveries obtainable with these procedures.

THE role played by that portion of the soil bases that can be extracted by neutral salts or weak acids or separated by electrodialysis is now well recognized in different branches of soil science. A knowledge of the base-exchange capacity as well as the amounts and the nature of the exchangeable bases present in the soil is indispensable in most studies dealing with either the physical and chemical behavior of the soil (*3*, *7*) or soil fertility (*2*, *9*, *12*). In view of small amounts of exchangeable bases usually found, especially in light sandy soils, the determination of the exchangeable bases is attended with considerable analytical difficulties necessitating the use of a rather large soil sample and a large volume of the extracting solution (*4*, *9*, *12*). The need for more rapid methods to permit periodic analyses of soils from experimental plots as well as in soil survey studies has led to the development of the rapid micromethods herein described. It is estimated that the time required to make the necessary routine determinations with these methods has been greatly reduced without much sacrifice of accuracy. Although the methods have been developed primarily for use on light sandy soils with low exchange capacities, they should be adaptable to heavier soils, in which case the analysis may be carried out on a 5- to 10-gram sample of soil.

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The exchangeable bases are extracted with 1 *N* ammonium acetate solution (*12*) and the exchange capacity is determined by distillation of the ammonia adsorbed by the soil (*7*, *12*). Following the evaporation of the ammonium acetate extract the organic matter and the ammonium salts are destroyed by digestion with nitric and hydrochloric acids, followed by ignition at 380° C. After evaporation with hydrochloric acid, the residue is dissolved in 10 ml. of 0.1 *N* nitric acid. The exchangeable bases are then determined directly in separate aliquots, all of the precipitations and washings being carried out in a 15-ml. centrifuge tube as shown in the accompanying diagrammatic scheme.

Apparatus

Electric muffle with a rheostat and a pyrometer.

A centrifuge that will accommodate 24 15-ml. centrifuge tubes.

A photoelectric colorimeter preferably provided with light filters.

Pyrex 15-ml. conical centrifuge tubes graduated to contain 13 ml. and numbered consecutively. Another set of tubes, graduated at 13 ml., somewhat more constricted at the tip, so that the outside diameter measured 10 mm. from the tip is less than 8 mm., is selected for use in the determination of magnesium.

Pyrex test tubes, 150 × 20 mm. graduated at 20 ml.; 125 × 15 mm. graduated at 11 ml. and additional tubes of this size without graduation.

A water bath with a removable rack to hold 48 centrifuge tubes can be made of sheet copper. Figure 1 shows details of construction. The removable rack permits cooling the tubes conveniently by immersion in a similar container filled with cold water. If the rack is properly constructed, the contents of the tube can be mixed by spinning the tube in the bath and allowing the solution to whirl inside the tube.

Another bath, consisting of a 1-liter beaker fitted with a sheet copper lid which supports a centrifuge tube holder and a thermometer, is used in the permanganate titration of calcium. The titration can be thus performed at constant temperature and the end point observed without removing the tube from the bath.

Glass stirring rods are illustrated in Figure 2. Type A is used for stirring the solutions in the centrifuge tubes as in precipitation of magnesium, whereas type B is employed in breaking up precipitates packed by centrifuging.

1-ml., 2-ml., 3-ml., and 10-ml. transfer pipets.

Reagents, Standard Solutions, and Calibration Curves

EXTRACTION AND DETERMINATION OF EXCHANGE CAPACITY. All chemicals, unless otherwise specified, are of reagent grade quality.

Ammonium acetate, 1 *N*, pH 7.0. Prepare sufficient volume, preferably in a Pyrex bottle, by mixing 70 ml. of ammonium hydroxide, specific gravity 0.90, and 58 ml. of acetic acid, 99.5 per

cent, per liter of solution desired. After cooling, adjust exactly to pH 7.0 and dilute to the mark.

Alcohol, 80 per cent, neutral. Mix 16 volumes of 95 per cent ethyl alcohol with 3 volumes of water and adjust to pH 7 with ammonium hydroxide, using bromothymol blue as the indicator. This preliminary neutralization is necessary to prevent hydrolysis (*4*, *12*) of the adsorbed ammonium. Neutralized methyl alcohol may be substituted.

Sodium chloride, 10 per cent. Dissolve in water ordinary commercial salt sufficiently free of ammonia and acidify with hydrochloric acid.

Standard 0.1 *N* sulfuric acid and standard 0.1 *N* sodium hydroxide, carbon dioxide–free.

Removal of Organic Matter and Ammonium Salts. Fuming nitric acid, specific gravity 1.49; hydrochloric acid, specific gravity 1.18; hydrogen peroxide 30 per cent; dilute hydrochloric acid (1 + 1); 0.1 *N* nitric acid.

Separation of Manganese, Iron, Aluminum, and Phosphorus. Ammonium chloride, 25 per cent. Dissolve 250 grams of salt and dilute to 1 liter with water.

Ammonium hydroxide, 0.6 *N*.

Bromine water. Saturated solution of bromine in water.

Ferric chloride, 1 mg. of iron per ml. Dissolve 1.22 grams of ferric chloride ($FeCl_3.6H_2O$) in 250 ml. of water containing 1 ml. of concentrated hydrochloric acid.

Sodium acetate, 10 per cent. Dissolve 50 grams of sodium acetate ($NaC_2H_3O_2.3H_2O$) in water and dilute to 500 ml.

Methyl red, 0.02 per cent. Triturate 0.04 gram of methyl red with 1.5 ml. of 0.1 *N* sodium hydroxide until the dye has dissolved and dilute to 200 ml. with water.

Determination of Calcium. Ammonium hydroxide, 2 *N*, saturated with calcium oxalate. Dilute 1 volume of ammonium hydroxide, specific gravity 0.90, with 6 volumes of water and saturate with calcium oxalate. Filter or siphon the clear solution before use.

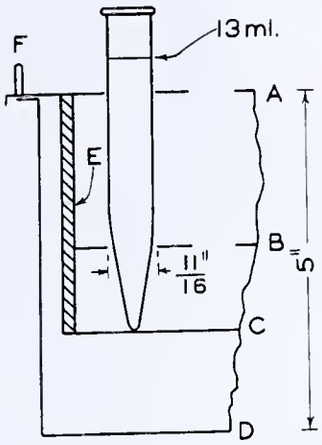


Figure 1. Section of Water Bath

A, *B*, *C*, removable rack; *D*, container; *E*, supporting rod; *F*, handle. Holes in *A* and *B* are 11/16 inch in diameter and 1 3/16 inches apart on centers; bottom of *C* is perforated to permit circulation; dimensions of bath 16 × 8 × 5 inches

Ammonium oxalate, 3 per cent. Dissolve 30 grams of ammonium oxalate [$(NH_4)_2C_2O_4.H_2O$] per liter of water.

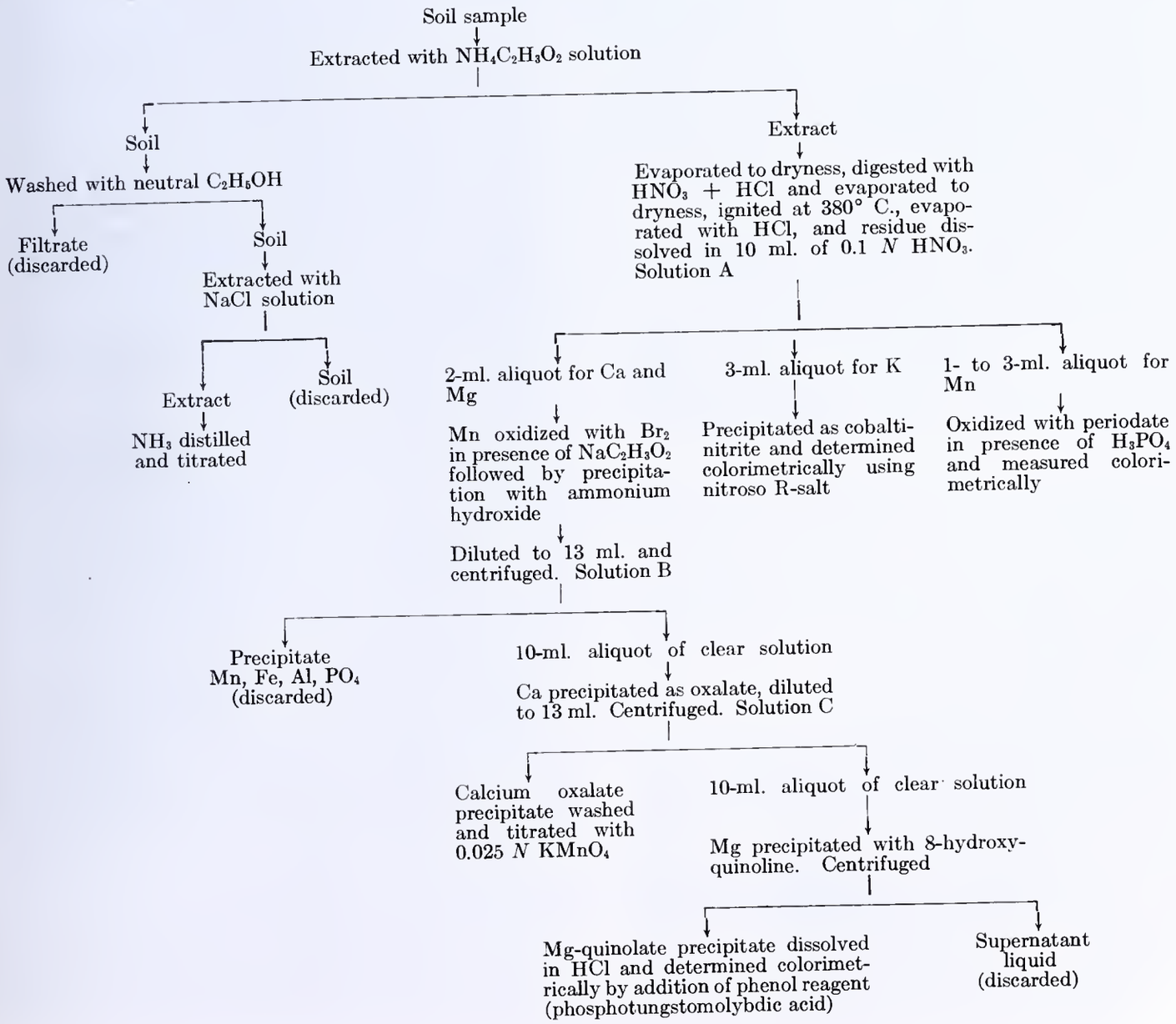
Hydrochloric acid, 0.5 *N*; sulfuric acid, 10 per cent.

Standard potassium permanganate, 0.025 *N* and 0.05 *N*. Dilute 0.1 *N* stock solution to 0.025 *N* and 0.05 *N* and standardize against sodium oxalate. The 0.05 *N* standard permanganate solution is used when the calcium oxalate precipitate is exceptionally heavy.

Determination of Magnesium. Ammonium hydroxide, concentrated, specific gravity 0.90; hydrochloric acid, 0.5 *N*.

Ammoniacal ammonium acetate, wash solution. To 200 ml. of 1 *N* ammonium acetate add 100 ml. of distilled water and 8 ml. of concentrated ammonium hydroxide.

8-Hydroxyquinoline, 2 per cent. This solution should be prepared freshly by dissolving 0.5 gram of 8-hydroxyquinoline in


Soil sample
Extracted with $NH_4C_2H_3O_2$ solution
Soil
Washed with neutral C_2H_5OH
Filtrate (discarded)
Soil
Extracted with NaCl solution
Extract
NH_3 distilled and titrated
Soil (discarded)
Extract
Evaporated to dryness, digested with HNO_3 + HCl and evaporated to dryness, ignited at 380° C., evaporated with HCl, and residue dissolved in 10 ml. of 0.1 *N* HNO_3. Solution A
2-ml. aliquot for Ca and Mg
Mn oxidized with Br_2 in presence of $NaC_2H_3O_2$ followed by precipitation with ammonium hydroxide
Diluted to 13 ml. and centrifuged. Solution B
3-ml. aliquot for K
Precipitated as cobaltinitrite and determined colorimetrically using nitroso R-salt
1- to 3-ml. aliquot for Mn
Oxidized with periodate in presence of H_3PO_4 and measured colorimetrically
Precipitate Mn, Fe, Al, PO_4 (discarded)
10-ml. aliquot of clear solution
Ca precipitated as oxalate, diluted to 13 ml. Centrifuged. Solution C
Calcium oxalate precipitate washed and titrated with 0.025 *N* $KMnO_4$
10-ml. aliquot of clear solution
Mg precipitated with 8-hydroxyquinoline. Centrifuged
Mg-quinolate precipitate dissolved in HCl and determined colorimetrically by addition of phenol reagent (phosphotungstomolybdic acid)
Supernatant liquid (discarded)

25 ml. of 95 per cent ethyl alcohol.

Phenol reagent (*5*). To 750 ml. of water in a 2-liter flask add 100 grams of sodium tungstate ($Na_2WO_4.2H_2O$), 20 grams of phosphomolybdic acid (20 $MoO_3.2H_3PO_4.48H_2O$), and 50 ml. of 85 per cent phosphoric acid. Boil for 2 hours, cool, and dilute to 1 liter with distilled water.

Sodium carbonate, 20 per cent. Dissolve 200 grams of the anhydrous salt in water, dilute to 1 liter, and filter if necessary.

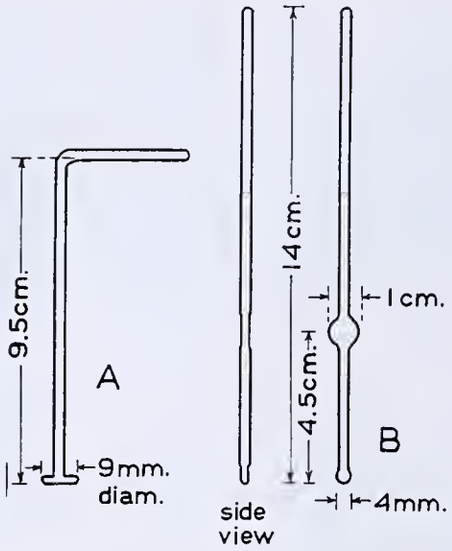


FIGURE 2. STIRRING RODS FOR USE IN 15-ML. CENTRIFUGE TUBES MADE FROM 3-MM. GLASS ROD

Standard Magnesium Quinolate Solution and Calibration Curve. Dissolve 0.15 gram of magnesium sulfate ($MgSO_4.7H_2O$) in 100 ml. of 10 per cent ammonium chloride solution, heat to 60–70° C., add 10 ml. of the 8-hydroxyquinoline reagent, and render the solution alkaline with 4 ml. of concentrated ammonium hydroxide. Digest for 10 minutes, collect the precipitate on a fritted-glass crucible, wash with hot dilute ammonium hydroxide (1 + 40), and dry at 140° C. Dissolve 0.0643 gram of the dried precipitate in 20 ml. of 0.5 *N* hydrochloric acid and dilute to 500 ml. One milliliter contains 0.01 mg. of magnesium. Introduce aliquots of this solution containing from 0.0025 to 0.06 mg. of magnesium into 50-ml. volumetric flasks, dilute with water to about 35 ml., and develop the color as directed in the method for magnesium. Plot the scale readings on the colorimeter against the concentration of the respective standards on semilogarithmic graph paper.

DETERMINATION OF POTASSIUM. Alcohol, 70 per cent. Dilute 500 ml. of 95 per cent ethyl alcohol with 180 ml. of water.

Nitroso R-salt, 0.5 per cent. Dissolve 0.5 gram of nitroso R-salt (disodium salt of 1-nitroso-2-hydroxy-3,6-naphthalenedisulfonic acid) in 100 ml. of water. When not exposed to direct sunlight the reagent is stable for several weeks.

Sodium cobaltinitrite, 25 per cent. Dissolve 25 grams of potassium-free trisodium cobaltinitrite in water, dilute to 100 ml., and filter. Cool the solution before use and keep in a refrigerator. Prepare freshly the necessary amount of this reagent as needed.

Sodium pyrophosphate, 5 per cent. Dissolve 5 grams of powdered sodium pyrophosphate ($Na_4P_2O_7.10H_2O$) in 100 ml. of water.

Sodium acetate, 2.5 *N;* sulfuric acid, 2.0 *N*.

Standard Potassium Solution and Calibration Curve. Dissolve 0.9533 gram of dried potassium chloride in 500 ml. of water. One milliliter contains 1 mg. of potassium. Dilute a portion of this stock solution to contain 0.5 mg. of potassium per ml. Measure out aliquots of the standard solutions ranging from 0.1 to 2.0 mg. of potassium into a series of 15-ml. centrifuge tubes, add 0.3 ml. of 1 *N* nitric acid, and dilute to 3 ml. with distilled water. Carry these standards through the procedure outlined for potassium and use the resultant data to plot the deflection-concentration curve.

DETERMINATION OF MANGANESE. Phosphoric acid, 85 per cent.

Sodium periodate or potassium periodate. The more soluble sodium metaperiodate is preferred to the potassium salt. L. H. Greathouse of this station's staff has suggested the use of periodic acid (H_5IO_6) now obtainable. Its high solubility and stability (*16*) afford the addition of a readily measured amount of a concentrated solution of this reagent.

Standard Manganese Solution and Calibration Curve. To 22.8 ml. of 0.1 *N* standard potassium permanganate in a 250-ml. Erlenmeyer flask add about 50 ml. of distilled water and a few drops of concentrated sulfuric acid. Heat to boiling and reduce the permanganate by the addition of sodium sulfite. Boil off the excess sulfur dioxide and dilute to 1 liter. One milliliter of this solution is equivalent to 0.025 mg. of manganese. Prepare a series of standards containing from 0.005 to 0.20 mg. of manganese in 11-ml. graduated test tubes, add 1 ml. of 85 per cent phosphoric acid, and proceed as directed in the determination of manganese. From the colorimeter readings plot the deflection-concentration curve.

Extraction and Determination of Exchange Capacity

Weigh a sufficient sample of soil to give 1 to 2 milliequivalents (see discussion of the methods), into either a funnel or a Gooch crucible fitted with filter paper, leach with about 225 ml. of 1 *N* ammonium acetate, using gentle suction if necessary, and catch the filtrate in a 250-ml. beaker. Usually a series of 24 samples is leached simultaneously. After leaching with ammonium acetate, place the beaker on a hot plate and evaporate the extract to dryness. Remove the excess of ammonium acetate from the soil by washing with 150 ml. of 80 per cent neutral alcohol. Drain well and leach again with 200 ml. of 10 per cent sodium chloride solution to replace the adsorbed ammonium. Transfer the sodium chloride extract to a Kjeldahl flask, add 150 ml. of water and 2 to 3 grams of calcium hydroxide, and distill the ammonia into 30 ml. of standard 0.1 *N* sulfuric acid. Collect 200 ml. of distillate and titrate the excess acid with standard 0.1 *N* sodium hydroxide.

For a 20-gram sample of soil, ml. of 0.1 *N* sulfuric acid consumed × 0.5 = exchange capacity in milliequivalents per 100 grams of soil.

Determination of Exchangeable Bases

DESTRUCTION OF ORGANIC MATTER AND AMMONIUM SALTS. Allow the residue from the evaporation of the ammonium acetate extract to cool, cover the beaker with a watch glass, and add slowly through the lip 5 ml. of fuming nitric acid and 1 ml. of concentrated hydrochloric acid. Warm on a hot plate until the reaction has subsided and the brown fumes are no longer given off. Cool a little, rinse the watch glass into the beaker, and evaporate the solution to dryness at low heat to prevent spattering. Continue to heat for about 10 minutes to dehydrate the salts, then place the beaker in an electric muffle at 150° to 200° C., heat to 380° C., and hold at this temperature for 10 to 15 minutes. Remove the beaker from the muffle and cool. Treat the residue with 3 ml. of dilute hydrochloric acid (1 + 1) to dissolve the oxides of manganese and iron, evaporate to dryness on a steam bath, and continue heating for 15 minutes longer to dehydrate silica. Cool and measure immediately from a buret 10 ml. of 0.1 *N* nitric acid into the beaker; stir with rubber policeman to loosen and dissolve the residue of salts. The solution should be colorless and clear except for a trace of silica, which is either allowed to settle out by transferring the solution into a small test tube (125 × 15 mm.) or centrifuged in a 15-ml. centrifuge tube. (If the precipitate is large, which is seldom the case, centrifuge and wash with hot 5 per cent nitric acid; combine the washings with the original solution, evaporate to dryness on a steam bath, and redissolve the residue in 10 ml. of 0.1 *N* nitric acid.) Designate this as solution A.

SEPARATION OF MANGANESE, IRON, ALUMINUM, AND PHOSPHORUS. Transfer a 2-ml. aliquot of solution A to a 15-ml. centrifuge tube for the determination of calcium and magnesium. Add 0.2 ml. of ferric chloride solution, 3 ml. of water, and 2 ml. of 10 per cent sodium acetate solution, mix the contents, and then add 1 ml. of 0.1 *N* sodium hydroxide and mix again. Place the centrifuge tube in a water bath at 95° C., add 1 ml. of bromine water, and maintain this temperature for at least 1 hour to flocculate manganese dioxide and to expel the excess of bromine. Then add 2 ml. of 25 per cent ammonium chloride solution and digest for about 15 minutes longer. Add 1 drop of methyl red and if the color of the indicator persists, indicating complete expulsion of bromine, remove the tube from the water bath, cool, add 0.6 *N* ammonium hydroxide from a buret until the color of the solution changes from a slightly red to a deep yellow, and then add 2 drops in excess. A rather constant amount, 0.5 ml., of ammonium hydroxide is usually required and may be added at one time. Make up to volume of 13 ml., add 5 drops in excess to allow for evaporation, mix the contents with a stirring rod, and digest in a water bath at 80° C. for 5 minutes to flocculate the precipitate. Centrifuge while hot for 10 minutes. Designate as solution B.

DETERMINATION OF CALCIUM. Pipet a 10-ml. aliquot of solution B into another centrifuge tube without disturbing the precipitate of manganese, iron, and aluminum. This is done best by holding the tube in front of a mirror. Add 0.5 ml. of 0.5 *N* hydrochloric acid and 0.9 ml. of water, and place in a water bath at 70° C. Mix the contents by spinning the tube, add 2 ml. of 3 per cent ammonium oxalate, mix thoroughly again, and digest for 30 minutes at 70° C. Remove the tube from the bath and let stand for 30 minutes. Make to volume, mix, and centrifuge for 15 minutes at 1700 r. p. m. It is seldom necessary to adjust the

Table I. Effect of Ignition Temperature, Ammonium Salts, Sulfates, and Other Ions on Recovery of Potassium by Proposed Method

Additions and Treatment[a]	K in Aliquot Found	K in Aliquot Calculated
	Mg.	*Mg.*
2.94 mg. K, 20 mg. Ca, evaporated with HNO_3–HCl, not ignited	0.87	0.88
2.94 mg. K, 20 mg. Ca, evaporated with HNO_3–HCl, ignited at 380° C.	0.86	0.88
2.94 mg. K, 20 mg. Ca, evaporated with HNO_3–HCl, ignited at 410° C.	0.86	0.88
2.94 mg. K, 20 mg. Ca, 2.0 mg. Mg, 0.5 mg. Mn, 1.0 mg. Fe, evaporated with HNO_3–HCl, ignited at 380° C.	0.87	0.88
30 mg. Ca, 250 mg. NH_4Cl, evaporated with HNO_3–HCl, ignited at 380° C.	0.00[b]	0.00
30 mg. Ca, 250 mg. NH_4Cl, 1.5 milliequivalents H_2SO_4, evaporated with HNO_3–HCl, ignited at 380° C.	0.00[b]	0.00
30 mg. Ca, 250 mg. NH_4Cl, 4.0 milliequivalents H_2SO_4, evaporated with HNO_3–HCl, ignited at 380° C.	4.0[c]	0.00
2.00 mg. K, 30 mg. Ca, 250 mg. NH_4Cl, evaporated with HNO_3–HCl, ignited at 380° C.	0.60[b]	0.60
2.00 mg. K, 30 mg. Ca, 250 mg. NH_4Cl, 1.0 milliequivalent H_2SO_4, evaporated with HNO_3–HCl, ignited at 380° C.	0.55[d]	0.60
2.00 mg. K, 30 mg. Ca, 250 mg. NH_4Cl, 4.0 milliequivalents H_2SO_4, evaporated with HNO_3–HCl, ignited at 380° C.	2.0[c]	0.60

[a] Calcium, magnesium, potassium, manganese, and iron added as chlorides. Evaporation with fuming nitric and hydrochloric acids and subsequent treatment carried out as outlined in procedure. Residue of salts taken up in 10 ml. of 0.1 *N* nitric acid and 3-ml. aliquot taken for potassium determinations.
[b] Test with Nessler's reagent showed complete expulsion of ammonium salts by treatment.
[c] Large amounts of ammonium found present after treatment.
[d] Complete expulsion of ammonium salts effected by treatment; low potassium result due to formation of the less soluble double salt $CaSO_4.K_2SO_4$.

volume before centrifuging, since 0.4 ml. of water is provided in excess to compensate for evaporation. Decant the clear supernatant liquid into a dry test tube and save for the determination of magnesium (solution C). Allow the centrifuge tube, inverted at about a 45° angle, to drain for several minutes on filter paper, add 5 ml. of 2 *N* ammonium hydroxide saturated with calcium oxalate, break up the precipitate by means of a stirring rod, wash the rod, and centrifuge for 15 minutes at 1700 r. p. m. Decant the solution, drain the tube, and discard the clear liquid. One washing is sufficient, provided the centrifuge tube has been drained properly each time after decantation. Add 5 ml. of 10 per cent sulfuric acid, heat to 70° C. in a water bath, and titrate with standard permanganate. If the titer is greater than 5 ml., add about 4 drops of concentrated sulfuric acid before completing the titration.

For a 20-gram sample of soil and 2-ml. aliquot of solution A, 1 ml. of 0.025 *N* potassium permanganate = 0.810 milliequivalent per 100 grams, or 325 pounds of calcium per acre-6-inches (2,000,000 pounds).

Determination of Magnesium. Introduce a 10-ml. aliquot of the solution from the calcium determination (solution C) into a 15-ml. centrifuge tube. Place the tube in a bath at 70° C., add 0.8 ml. of 2 per cent alcoholic solution of 8-hydroxyquinoline, mix immediately by stirring, and then add 0.4 ml. of concentrated ammonium hydroxide from a buret. Stir vigorously for 1 minute, or longer if the amount of magnesium is extremely small, until full turbidity develops. Wash the stirring rod with a few drops of water and replace the centrifuge tube in a water bath at 70° C. for 10 minutes to flocculate the precipitate. If a number of magnesium determinations are to be carried out simultaneously, set the centrifuge tubes aside after precipitation, until the magnesium in the last tube has been precipitated, then replace the tubes in a bath at 70° C. for 10 minutes. After digestion for 10 minutes, cool by immersing the centrifuge tubes in a bath at about 25° C., and allow to stand for 45 minutes to assure complete precipitation of magnesium; then add 0.5 ml. of 95 per cent ethyl alcohol slowly down the sides of the centrifuge tube, rotating the tube at the same time in order to wash down the precipitate and to form a layer of alcohol on the surface of the solution. Centrifuge for 15 minutes at 1700 r. p. m. and by using gentle suction draw off 2 to 3 ml. of the clear liquid to remove the layer of alcohol. Decant carefully and discard the solution; wipe the mouth of the tube with filter paper, add 5 ml. of ammoniacal ammonium acetate, wash solution down the sides of the tube, break up the precipitate with a stirring rod, and wash the rod into the tube; add 0.5 ml. of alcohol down the sides of the tube to prevent creeping of the precipitate, and centrifuge for 15 minutes at 1700 r. p. m. Draw off the layer of alcohol, decant, and repeat the washing once more as directed above. Dissolve the precipitate in 4 ml. of 0.5 *N* hydrochloric acid, dilute to 13 ml. with water, stopper, and mix. Transfer a 2-ml. aliquot (use 1 ml. if solution is distinctly yellow) to a 50-ml. volumetric flask, and add about 35 ml. of water, 5 ml. of 20 per cent sodium carbonate, and 3 ml. of phenol reagent, mixing the contents after each addition. Place the flask in boiling water for 1 minute, remove from the bath, and cool after 15 minutes. Make to volume, mix, and read in the colorimeter.

For a 20-gram sample of soil, and 2-ml. aliquot of solution A, 1 mg. of magnesium found precipitated = 3.48 milliequivalents per 100 grams, or 845 pounds of magnesium per acre.

Determination of Potassium. Pipet a 3-ml. aliquot of solution A into a 15-ml. centrifuge tube. If smaller aliquot is used, dilute to 3 ml. with 0.1 *N* nitric acid. Add 1 ml. of sodium cobaltinitrite reagent and mix the contents thoroughly by whirling the tube. Let stand in the refrigerator for 1 hour, then add 4 ml. of 70 per cent alcohol, stir thoroughly with a stirring rod, wash the rod with alcohol, and centrifuge for 15 minutes at 1700 r. p. m. Decant the supernatant liquid, drain the tube for several minutes on filter paper, add 5 ml. of 70 per cent alcohol down the walls of the tube, break up the precipitate with a stirring rod, wash the rod with 1 ml. of alcohol, and centrifuge for 10 minutes at 1700 r. p. m. Decant the clear solution, allowing the tube to drain for several minutes, and repeat the washing with 5 ml. of 70 per cent alcohol. Dissolve the precipitate in 5 ml. of 2 *N* sulfuric acid by placing the tube in a bath at about 70° C. After the precipitate in the bottom of the tube has been dissolved, add 5 to 7 ml. of water and heat for another 5 to 10 minutes to dissolve any adhering precipitate on the sides of the tube. Cool, dilute to 13 ml., stopper, and mix thoroughly. Introduce a 1-ml. aliquot of the solution into a test tube (150 × 20 mm.) graduated at 20 ml. Add 1 ml. of 5 per cent sodium pyrophosphate solution, dilute to volume with water, add 1 ml. of 2.5 *N* sodium acetate solution, mix, and then add 2 ml. of 0.5 per cent solution of nitroso R-salt; mix well again, and read in the colorimeter after 15 minutes.

For a 20-gram sample of soil and 3-ml. aliquot of solution A, 1 mg. of potassium found precipitated = 0.426 milliequivalent per 100 grams, or 333 pounds of potassium per acre.

Determination of Manganese. Transfer a 1- to 3-ml. aliquot of solution A, depending upon the amount of manganese present, which can be estimated from the previous separation of manganese, to a test tube graduated at 11 ml. Add 1 ml. of 85 per cent phosphoric acid, dilute to volume with water, adding 0.3 ml. to allow for evaporation, and mix with a glass stirring rod. Place in a water bath at 95° C., add about 50 mg. of sodium periodate, mix thoroughly again with a glass rod, and leave in the bath for 1 hour to assure full development of the color. Cool, make to volume if necessary, mix, and read in the colorimeter.

For a 20-gram sample of soil and 3-ml. aliquot of solution A, 1 mg. of manganese found = 0.607 milliequivalent per 100 grams, or 333 pounds of manganese per acre.

Discussion of Methods

To provide the amounts of the various constituents in the range that can be handled conveniently by the present methods, the weight of the soil sample taken for extraction should be varied with the exchange capacity which can be roughly estimated from its texture and organic matter content. A 20-gram sample is taken if the exchange capacity is between 5 and 10 milliequivalents, a 10-gram sample if the exchange capacity is between 10 and 20 milliequivalents, or sufficient sample to give 1 to 2 milliequivalents. The aliquots suggested are based upon the average distribution of exchangeable bases found in the majority of soils.

The ignition at 380° C., following the treatment of the residue left after the evaporation of the ammonium acetate extract with a mixture of nitric and hydrochloric acids, assures complete expulsion of organic matter and ammonium salts without loss of the constituents to be determined. Prolonged heating and higher temperature should be avoided to prevent volatilization of the alkalies. The heating operation usually requires less than 1 hour from the time the beakers are placed in the muffle at about 150° C. Ammonium sulfate is

Table II. Effect of Iron, Aluminum, Manganese, and Phosphate on Determination of Calcium and Magnesium

Soil Used	Additions[a]	Soil Sample Weight	P Found in NH_4OAc Extract	Ca Found[b]	Mg Found[b]
		Grams	*Lb./acre*	*Mg.*	*Mg.*
None	2.20 mg. Mg	..	..	0.0	0.28
None	20.4 mg. Ca, 2.20 mg. Mg	..	..	3.3	0.28
None	20.4 mg. Ca, 2.20 mg. Mg, 0.50 mg. Mn	..	..	3.3	0.28
Parkwood clay loam	20.4 mg. Ca, 2.20 mg. Mg, 0.50 mg. Mn, 1.5 mg. Fe, 1.5 mg. Al	..	..	3.3	0.28
Parkwood clay loam	0.3 mg. Fe to remove PO_4[c]	25	6	8.6	0.35
Parkwood fine sandy loam	None	25	6	8.5	0.34
Parkwood fine sandy loam	0.3 mg. Fe added to remove PO_4[c]	50	36	9.2	0.18
Norfolk fine sand	None	50	36	9.2	0.19
Norfolk fine sand	0.3 mg. Fe added to remove PO_4[c]	50	25	4.9	0.28
Bladen fine sandy loam	None	50	25	4.8	0.29
Bladen fine sandy loam	0.3 mg. Fe added to remove PO_4[c]	50	18	5.2	0.22
	None	50	18	5.2	0.21

[a] Synthetic solutions as well as soil extracts were carried through procedure and salts finally dissolved in 10 ml. of 0.1 *N* HNO_3; 2-ml. aliquots taken for removal of Mn, Fe, Al, and PO_4 prior to determination of calcium and magnesium in accordance with outlined procedures.
[b] Calcium and magnesium found precipitated in aliquots taken.
[c] Ferric chloride added to 2-ml. aliquot prior to separation of Mn, Fe, Al, and PO_4; subsequent test showed complete removal of PO_4.

decomposed completely as readily as ammonium chloride and ammonium nitrate by this treatment, provided the amount of sulfate is not in excess of the equivalent of the bases present, a condition, of course, never attained. Data are presented in Table I to show the effect of the ignition temperature and sulfate on the volatilization of ammonium salts and subsequent recovery of potassium from synthetic solutions by the proposed method.

The efficacy of this gentle ignition to destroy the last traces of organic matter depends upon the presence of salts of nitric acid, especially of magnesium nitrate. Occasionally, with soils extremely low in exchangeable bases (acid sandy subsoils) some carbon is formed during the ignition process and resists further ashing at 380° C. even upon repeated evaporation with nitric acid and ignition. This carbon can be removed by digestion with 1.0 ml. of 30 per cent hydrogen peroxide. If much manganese is present, the residue from the ignition will be dark, owing to manganic oxide which should not be mistaken for carbon. While the preliminary treatment of the residue left upon evaporation of the ammonium acetate extract is rapid, it provides for complete destruction of organic matter and ammonium salts, leaving readily soluble salts which are dissolved in a small volume of 0.1 *N* nitric acid suitable for direct determination of calcium, magnesium, potassium, and manganese. Some difficulty might be experienced in dissolving the residue of salts in 10 ml. of 0.1 *N* nitric acid in the case of alkali soils high in sulfates due to the relatively small solubility of calcium sulfate.

Prior to the precipitation of manganese by bromine, the addition of 2 ml. of 10 per cent sodium acetate solution and 1 ml. of 0.1 *N* sodium hydroxide to the 2-ml. aliquot buffers the solution to pH 5.7, which is sufficiently high to prevent dissolution of the manganese dioxide. If an aliquot of only 1 ml. of solution A is used, the addition of sodium hydroxide should be omitted. Both ammonium chloride and ammonium hydroxide are added after the excess of bromine has been expelled to preclude oxidation of the ammonium salts and increase in the acidity of the solution. Further digestion in the presence of ammonium chloride assures the removal of the last trace of bromine, which would otherwise destroy the indicator to be used later. The pH of solution B after centrifuging the precipitate of manganese, iron, and aluminum is usually about 6.8. If iron and aluminum are present in excess of the amount requisite for the combination with phosphorus as in heavy clay soils, the addition of ferric chloride should be omitted. Many sandy soils that have received continuous fertilization with phosphatic fertilizers often show only traces of iron and aluminum but as much as 30 pounds of phosphorus per acre upon leaching with 1 *N* ammonium acetate. While this amount of phosphorus was not found to interfere in the determination of calcium and magnesium as shown in Table II, it is a safe practice to provide for its removal when dealing with such soils. Because of the small amounts of manganese, iron, and aluminum extracted by ammonium acetate solution, it has not been found necessary to wash or reprecipitate the ammonia precipitate. Table II shows the results relative to the effect of manganese, iron, aluminum, and phosphate on the determination of calcium and magnesium by the proposed methods.

To lessen coprecipitation of magnesium, calcium is precipitated at approximately pH 5.0.

An attempt was made to determine magnesium by means of Titan yellow, but the method as previously reported (*10*) was not found applicable to the present scheme. Magnesium is, therefore, precipitated by 8-hydroxyquinoline and determined colorimetrically by the method of Yoshimatsu (*17*) which is based on the reduction of phenol reagent (phosphotungstomolybdic acid) by hydroxyquinoline. Experiments conducted to study the effect of the oxalate showed that, although the presence of oxalate seems to inhibit complete precipitation of magnesium as recently reported by Alexander and Harper (*1*), if the solution is not stirred sufficiently, excellent recoveries even of small amounts of magnesium were obtained from solutions containing large amounts of ammonium oxalate when the solutions were stirred vigorously for at least one minute after the addition of the reagents. The destruction of the oxalate (*1*) prior to the precipitation of magnesium with 8-hydroxyquinoline is, therefore, unnecessary. Scratching the sides of the tube with the stirring rod to induce the precipitation of magnesium is not only unnecessary but leads to breakage of the tubes in the centrifuge.

The conditions for precipitation of magnesium outlined in the procedure have given consistently good results. Considerable difficulty was experienced at first in washing the precipitate when using the centrifuge technique. The precipitate is light, does not pack wel upon centrifuging, and tends to creep along the sides of the centrifuge tube, which makes it difficult to decant the solution without some loss of the precipitate. Hoffman (*6*) has designed a special centrifuge tube and proposed the use of a 50 per cent alcohol solution saturated with magnesium quinolate for washing the precipitate, as well as layering the solution with 95 per cent alcohol to prevent creeping of the precipitate. Although the precipitate shows little tendency to escape from alcoholic solutions, it does not pack well when centrifuged from 50 per cent alcohol solution and tends to break away during the decantation process. The procedure finally adopted— i. e., washing the precipitate with ammoniacal ammonium acetate solution and layering with 95 per cent ethyl alcohol—has been found satisfactory and admits the use of an ordinary 15-ml. centrifuge tube. For this purpose, however, it is advisable to select centrifuge tubes that are somewhat more

Table III. Recoveries of Added Calcium, Magnesium, Potassium, and Manganese from Soil Extracts[a]

Soil Type		Exchange Capacity	Soil Sample Weight	Calcium		Magnesium		Potassium		Manganese	
				Found	Calculated	Found	Calculated	Found	Calculated	Found	Calculated
		Milliequivalents/ 100 g.	*Grams*	*Pounds per acre (2,000,000 pounds of soil)*							
Norfolk fine sand	A	2.05	50	174		8		52		1.6	
	B		50	944	974	68	68	110	112	5.5	5.6
Eustis fine sand	A	2.85	50	435		25		80		4.7	
	B		50	1200	1235	86	85	141	140	8.6	8.7
Blanton fine sand	A	4.20	50	840		66		91		4.0	
	B		50	1650	1640	123	126	148	151	7.9	8.0
Orlando fine sand	A	6.60	50	362		17		125		4.7	
	B		50	1165	1162	77	77	182	185	8.7	8.7
Portsmouth fine sandy loam	A	9.35	20	1650		187		153		1.3	
	B		20	3620	3650	324	337	303	303	11.6	11.3
Parkwood clay loam (calcareous)	A	7.85	20	8380		405		153		5.0	
	B		20	10500	10380	560	555	296	303	15	15
Bladen clay loam	A	21.6	10	5020		693		760		23	
	B		10	8800	9020	975	993	1050	1060	42	43
Miami silt loam (Ohio)	A	9.60	20	2680		538		176		25	
	B		20	4750	4680	695	688	333	326	35	35

[a] Ammonium-acetate leachings from duplicate samples were composited and then divided equally between A and B; A represents initial soil composition; B received following additions: 20.0 mg. Ca, 1.50 mg. Mg, 1.50 mg. K, and 0.100 mg. Mn as chlorides.

constricted at the tip. The precipitate shows much less tendency to break away from the bottom of the tube and proper drainage of the tubes is facilitated if the layer of alcohol is first drawn off before decanting the supernatant liquid.

The precipitation of potassium in the presence of nitric acid as recently shown by Wilcox (*14*) offers several advantages, one of which is that this acid retards the nitrite decomposition. Complete precipitation of potassium takes place in one hour if the temperature of the solution is maintained at 10° C. The amount of potassium is finally determined indirectly by the colorimetric cobalt procedure, using the nitroso R-salt (*8*, *13*). In developing the cobalt color with this reagent it is important that the pH of the solution be above 4. The color fails to develop below pH 3 but develops slowly between pH 3 and 4. Between pH 4 and 5 the color develops rapidly and the intensity is practically constant; above pH 5 the intensity of the color increases gradually with increase in pH. The addition of 1 ml. of 5 per cent solution of sodium pyrophosphate and 1 ml. of 2.5 *N* sodium acetate solution to the 1-ml. aliquot of the solution of the cobaltinitrite precipitate buffers the solution to pH 5.0. Sodium pyrophosphate was found effective in preventing the interference from small amounts of iron that might precipitate as the phosphate in the cobaltinitrite precipitation and would react subsequently with the nitroso R-salt, imparting a greenish cast to the color developed.

In the determination of manganese by the periodate method of Willard and Greathouse (*15*) the oxidation was first carried out in 5 per cent sulfuric acid solution, but since this concentration of acid was inadequate to prevent the precipitation of larger amounts of manganese, and in view of the failure of the small amounts of manganese to develop the color at higher concentrations of acid as shown by Richards (*11*), phosphoric acid was substituted for sulfuric acid. Difficulties encountered due to precipitation of calcium sulfate have been obviated also by substituting phosphoric acid. No fading of the color or brown coloration due to precipitation of manganese has ever been observed with the use of phosphoric acid, except in several instances when an attempt was made to decolorize the yellowish tint of the solution with ammonium persulfate before the addition of periodate. In such cases the permanganate color showed a brownish tint and the solutions developed a slight turbidity due to precipitation of manganese. The addition of persulfate to destroy traces of organic matter at this stage, even a few minutes prior to the addition of periodate, is likely to cause precipitation of manganese, especially at higher concentrations, and should be avoided if the oxidation is carried out under the conditions specified in the procedure. Since an excess of periodate is added, the small amount of chloride present does not interfere and need not be removed.

Comments

The amount of exchangeable hydrogen in acid soils is computed by subtracting the sum of the exchangeable bases, excluding hydrogen, from the exchange capacity. Because of the appreciable solubility of calcium carbonate in ammonium acetate solution, the exchangeable calcium content of saturated soils containing an excess of calcium carbonate is also obtained by difference—i. e., by subtracting the sum of the bases, excluding calcium, from the exchange capacity. Because of the small amounts of exchangeable sodium found in sandy soils, the determination of sodium has been dispensed with in routine analyses.

The analytical results listed in Table III show typical recoveries obtained by the foregoing methods when known amounts of calcium, magnesium, potassium, and manganese were added to the ammonium acetate extracts of several different soils. The accuracy, which will vary somewhat with the amounts of the various constituents present, is within ±3 per cent. Blank determinations should be made from time to time on all reagents. A synthetic soil solution made to approximate the average composition of the extracts is often carried through the procedure simultaneously with a series of determinations.

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Detection and Titration of Chromate in Blood Iodine Determinations

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IN CHROMIC acid methods for determination of iodine in blood the organic matter is oxidized with a mixture of chromic and sulfuric acids. The iodine remains as iodic acid, which is reduced and distilled with or without aeration (*2, 4, 5*). There are 7 to 10 grams of chromium in the flask, from which a microgram or less of iodine is to be distilled. Should a trace of chromium be carried over, it would be oxidized in the next step, forming chromate. A study of factors affecting the reactions between chromate or iodate and potassium iodide has enabled the authors to titrate iodate and chromate in the presence of one another. (No attempt is made to distinguish between chromate and dichromate in this paper, since the acidities vary and the dilutions are so great that the solutions are practically colorless.) The procedure is of obvious value in testing apparatus and methods, even if it is not permanently included in the technique.

TABLE I. 0.001 *N* THIOSULFATE REQUIRED TO TITRATE IODINE LIBERATED BY IODATE OR DICHROMATE

(Iodide concentration, acidity, and time as variables. Room temperature, 24° to 28°)

	2 Cc. of 0.00005 *N* Potassium Iodate Acidified with:							
25 Per Cent Potassium Iodide	0.12 Cc. of 85 Per Cent Phosphoric Acid		0.1 Cc. of *N* Sulfuric Acid		0.2 Cc. of Glacial Acetic Acid		0.2 Cc. of Acetic Acid-Acetate Buffer	
	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.
Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
0.01	0.095	0.093	0.086	0.084	0.082	0.084	0.023	0.047
0.02	0.097	0.100	0.095	0.095	0.089	0.095	0.063	0.089
0.04	0.100	0.102	0.100	0.097	0.097	0.100	0.099	0.100
0.08	0.102	0.101	0.100	0.101	0.101	0.104	0.101	0.101
0.16[a]	0.104	0.104	0.102	0.101	0.105	0.105	0.102	0.105
	2 Cc. of 0.00005 *N* Potassium Dichromate Substituted for Iodate							
0.01			0.000	0.000	0.000	0.000	0.000	0.000
0.02					0.000	0.000	0.000	0.000
0.04							0.000	0.000
0.08		0.105					0.000	0.000
0.16[a]	0.106	0.108	0.103	0.103			0.000	

[a] The observation that high concentrations of iodide produce unfavorable changes in the starch-iodine color (*1*) was confirmed.

Titration of Iodate

The reaction of minute amounts of iodate with iodide goes to completion if either the acidity or the iodide concentration sufficiently exceeds a certain minimum. Trevorrow and Fashena (*5*) kept the acidity low, and recommended the use of 10 mg. of potassium iodide per cc. in the final titration. Matthews, Curtis, and Brode (*2*) used only 1.5 mg. of potassium iodide per cc., but titrated at high acidity.

Keeping either the acidity or the iodide concentration low minimizes interference from chromate (Table I). The recorded values are averages of a series of titrations. In the absence of a blank, the theoretical value should be 0.100 cc. The limit of accuracy in judging the end point was about 0.005 cc. From the lowest concentration of iodide no iodine was liberated by dichromate in 5 minutes, even though sulfuric acid was used. [Acidity alone did not readily decompose the potassium iodide which was used (Mallinckrodt's analytical reagent, granulated). Contrary to statements in the literature, 2 cc. of normal sulfuric acid, on addition of more than 1 mg. of potassium iodide per cc., did not liberate iodine at once, or even after several minutes.] Under the same conditions the reaction between iodate and iodide went almost to completion. A better differentiation was made by using acetic acid or an acetic acid–acetate buffer. In fact, if one uses the buffer and 0.04 cc. of 25 per cent potassium iodide the 2 cc. of solution may be yellow with chromate, yet no detectable amount of iodine will be liberated, while under the same conditions iodate reacts quantitatively. The minimum acidity required for a rapid reaction between iodate and iodide is, however, not far below that obtained with acetic acid alone, so that acetate must not be added indiscriminately.

Detection and Titration of Chromate

During several years of experience with various chromic acid methods the authors frequently encountered indefinite end points in the final titration. In blank determinations the starch-iodine color appeared after about one minute, but it reappeared again and again if titration with thiosulfate solution was attempted. Addition of oxalate or tartrate changed this slow liberation of iodine to instantaneous liberation of a larger amount, which then titrated sharply. These findings were duplicated with known chromate. Recognized precautions against passage of chromium into distillates had, of course, been observed—i. e., digests were adequately heated to expel chloride, blanks were run without chloride, and distilling outfits were equipped with refluxing bulbs and entrainment traps.

Interference of oxalate and tartrate in precipitations of trivalent chromium is commonly ascribed to formation of complexes. The same explanation is advanced for the striking effect of oxalate on oxidation of cuprous copper by iodine (*3*). The similar effect of oxalate on reduction of hexavalent chromium, which the authors observed, is conveniently demonstrated by adding a few milligrams of oxalate or oxalic acid to 2 cc. of 0.00005 *N* dichromate solution to which 0.05-cc. portions of 25 per cent potassium iodide, 1 *N* sulfuric acid, and starch indicator have previously been added. As little as 0.2 microgram of iron also catalyzes the reaction under the same conditions.

Under conditions intended to be identical, chromium may or may not appear in distillates. Whether its presence matters or not depends upon the amount, acidity, iodide concentration, presence of catalysts or inhibitors, and other factors. The vacant spaces in Table I represent conditions under which no reproducible values could be obtained because so many factors affected the length of the induction period and the rate of the reaction.

Procedure

After carrying out the oxidation with chromic and sulfuric acids, reduce with phosphorous acid and distill, using 10 cc. of 0.1 per cent potassium carbonate in the receiver. Acidify the distillate with 0.3 cc. of 1 *N* sulfuric acid. Oxidize with bromine and evaporate to 2 cc. Cool. Add 0.2 cc. of buffer, 0.05 cc. of 25 per cent potassium iodide, and 1 drop of 1 per cent starch. Titrate the iodine liberated by iodate with 0.001 *N* thiosulfate, then add several drops of 1 *N* sulfuric acid and about 3 mg. of recrystallized oxalic acid. If chromate is present it will liberate iodine immediately and quantitatively, so that one can titrate again to the starch end point.

The amounts of sulfuric acid and buffer are adjusted so that the solution is certain to be acid during oxidation with bromine and the potassium acetate in the buffer will suffice to convert the excess of sulfuric acid to acetic acid.

The 25 per cent potassium iodide solution is freshly prepared each time by dissolving 1 gram of the salt in 3.7 cc. of iodine-free water. The buffer is prepared by one-twelfth neutralization of glacial acetic acid with a saturated solution of potassium carbonate that has been washed with iodine-free alcohol. Let us suppose that the glacial acetic acid is 17.4 N, and the washed potassium carbonate solution 10.8 N. To 10 cc. of glacial acetic acid one would add $\frac{10 \times 17.4}{12 \times 10.8}$ or 1.34 cc. of the carbonate solution.

Summary

The reaction between minute concentrations of chromate or dichromate and iodide is influenced by many factors, notably acidity, iodide concentration, catalysts such as iron, and ions which form complexes with chromium, such as oxalate and tartrate.

Conditions were found under which the reaction between iodate and iodide goes to completion long before liberation of iodine by chromate can be detected. This made it possible to titrate iodate, and, after addition of acid and oxalate, to titrate chromate present in the same solution.

The procedure is of value in testing apparatus and methods for determination of iodine in blood, and in preventing returning end points when these are due to small amounts of chromate.

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A Method of Measuring Contact Angles

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WHEN a liquid drop lies on a horizontal solid plate, the area of contact between liquid and solid depends on the contact angle, θ, between air, liquid, and solid, and on the shape and volume of the drop and the surface tension and density of the liquid. The relation between these factors and the area of contact is generally too complicated to allow a calculation of θ from the size of the area. If, however, the drop is very small and very nearly circular, the diameter, Δ_0, of the basis of the drop is a function only of its volume, v, and of θ:

$$\frac{\Delta_0^3}{v} = \frac{24 \sin^3\theta}{\pi(2 - 3\cos\theta + \cos^3\theta)} \qquad (1)$$

This equation is arrived at in the following manner.

The surface tension tends to give the drop the shape of a part of a sphere (since a sphere has the smallest relative surface). Gravitation on the other hand tends to flatten the drop. The actual shape of the drop is determined by the simultaneous action of both these forces. The effect of the surface tension increases when v decreases, since the ratio of surface to volume increases as well; and the effect of gravitation decreases with v since the level differences in small drops are small. Therefore very small drops are practically spherical segments, and Equation 1 is the geometrical relation between the diameter, Δ_0, of the base of the segment, its volume, v, and the angle, θ, between the base and the curved surface.

If the drops are larger than, say, 0.0001 ml., the diameter, Δ, of the circle of contact is appreciably larger than the value, Δ_0, which it would have had in the absence of gravitation. The ratio $\frac{\Delta^3}{v}$ has, therefore, to be measured for several values of v and then extrapolated to $v = 0$. A curve for the relation between Δ and v is given by Wark (*4*).

In order to make the extrapolation to $v = 0$ feasible, small drops—e. g., 0.001, 0.002, 0.004, and 0.008 ml.—have to be used. They are driven out from a microsyringe. The Agla micrometer syringe of Burroughs Wellcome & Co., London, was found very convenient for determining the volume of such small drops. It is supplied with a steel needle which is not wetted by water and, therefore, delivers drops more readily than a micropipet of glass.

The diameter, Δ, of the contact circle is measured after the evaporation of a drop. After evaporation drops leave a mark on the solid surface which may be due to corrosion of the surface by the liquid, deposition of the solutes present in the liquid, or settling of particles suspended in it. If the marks are not clear enough, this third effect may be artificially increased by dusting ignited talc onto the drop surface; it slides down the slopes of the drop and forms a white ring round its boundary.

If the volumes recommended above are used, Δ has values between 0.05 and 0.5 cm. The diameter of the circular marks can be determined using any method suitable for such lengths. A microscope provided with eyepiece micrometer scale (for small values of Δ) and a mechanical stage (for large values of Δ) is most convenient. A total magnification of 20 diameters is sufficient.

The precision of the value obtained for $\frac{\Delta_0^3}{v}$ is limited chiefly by the deviation of the mark from an ideal circle. As no

Table I. Ratio, Δ_0^3/v, for Various Values of Contact Angle θ

θ Deg.	$\frac{\Delta_0^3}{v}$	Change of Δ_0^3/v per 1°	θ Deg.	$\frac{\Delta_0^3}{v}$	Change of Δ_0^3/v per 1°
30	18.54	0.62	92.5	3.575	0.094
32.5	17.00	0.54	95	3.34	0.090
35	15.64	0.48	97.5	3.12	0.086
37.5	14.45	0.42	100	2.90	0.082
40	13.40	0.38	102.5	2.69	0.080
42.5	12.46	0.34	105	2.495	0.076
45	11.62	0.30	107.5	2.305	0.073
47.5	10.87	0.28	110	2.123	0.070
50	10.18	0.25	112.5	1.948	0.067
52.5	9.55	0.23	115	1.781	0.064
55	8.97	0.21	117.5	1.622	0.061
57.5	8.44	0.20	120	1.470	0.058
60	7.94	0.19	122.5	1.326	0.055
62.5	7.475	0.17	125	1.189	0.052
65	7.04	0.16	127.5	1.059	0.048
67.5	6.635	0.15	130	0.938	0.046
70	6.25	0.145	132.5	0.823	0.042
72.5	5.89	0.14	135	0.717	0.040
75	5.55	0.13	137.5	0.618	0.036
77.5	5.22	0.12	140	0.527	0.033
80	4.92	0.12	142.5	0.444	0.030
82.5	4.62	0.11	145	0.369	0.027
85	4.34	0.107	147.5	0.302	0.024
87.5	4.075	0.102	150	0.242	
90	3.82	0.098			

surface (under ordinary conditions) is rigorously uniform, the diameter of the contact mark varies from point to point. If the average diameter is wrong by 1 per cent the error of the ratio $\frac{\Delta^3}{v}$ is 3 per cent.

When the value of $\frac{\Delta_0^3}{v}$ is measured or extrapolated, θ may be calculated using Table I, which gives the values of the function $\frac{24 \sin^3\theta}{\pi(2 - 3\cos\theta + \cos^3\theta)}$ for 49 values of θ. For non-tabulated values of $\frac{\Delta_0^3}{v}$ a linear interpolation is sufficient.

Table I also shows that a 3 per cent error in $\frac{\Delta_0^3}{v}$ causes an error of about 1 per cent (or less) in the value of θ. If the solid surface is very good, like those of built-up multilayers (*2*), $\frac{\Delta_0^3}{v}$ can often be determined within ± 0.6 per cent and θ therefore calculated within $\pm 0.2°$.

In a recent paper Benedetti-Pichler and Rachele (*1*) estimated the size of minute droplets of water on cellulose nitrate by measuring the diameter of the circle of contact. They found that the ratio $\frac{\Delta_0^3}{v}$ was about 9; Table I shows then that the contact angle between air, water, and cellulose nitrate was about 55°.

The method here described was used for drops of water on built-up multilayers of soaps (*2*), on lacquered tin plate (*3*), and on glass plates.

When compared with the usual method of a direct measurement of θ this method is indicated when determination of volume and length is simpler than that of angle. It has also another advantage. When the profile of a drop is observed, the angle measured is that for one point only; the same drop viewed from another side may have a different shape and form a different contact angle. The area of contact between drop and solid is a measure of an average of all the contact angles present along the circumference of the drop, and the shape of the mark shows how constant (or otherwise) is the contact angle along this circumference.

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A Wet-Combustion Micromethod for Determination of Carbon and Hydrogen

Iodic Acid as an Oxidant for Wet Combustion

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CHRISTENSEN and Facer (*1*) have recently published a wet-combustion method using iodate as the oxidant for the determination of carbon and hydrogen. Previous experience has convinced the writers that the use of iodate in this connection is best adapted to micro work. Since a method which would handle small samples (2 to 5 mg.) is highly desirable in many cases, the possibility of devising such a procedure was studied.

In connection with these studies the method of Christensen and Facer was modified. Changes in the procedures for determining the evolved carbon dioxide and the method of treating the unreduced iodate, and a number of other modifications resulted in a considerable saving of time.

Although these wet-combustion methods are simple, permit intermittent operation, and employ the minimum of apparatus, their success depends upon the use of iodic acid as an oxidant for the organic material. This reagent has given excellent results for many organic compounds, yet some compounds under the conditions specified for analysis cannot be quantitatively oxidized by iodic acid. It was the opinion of the authors that much more information must be obtained regarding the behavior of this reagent toward all kinds of organic compounds (volatile, insoluble, etc.) before a wet-combustion method involving iodic acid could be established. It was possible that further study of the limitations of iodic acid as an oxidant in wet combustions might afford a criterion for predicting the behavior of a given compound when treated with iodic acid. For these reasons the following investigation was undertaken.

Reagents

Sulfuric acid, 96 per cent. It is important that the sulfuric acid be made as free as possible from organic matter. This was accomplished by adding 50 ml. of Superoxol dropwise to 200 ml. of hot concentrated sulfuric acid. This mixture was just brought to boiling and the heating continued until the sulfuric acid attained approximately its original volume.

Barium hydroxide, approximately 0.1 *N*, was prepared and protected by a soda-lime tube.

Hydrochloric acid, approximately 0.05 *N*, was prepared and standardized with potassium iodate.

Thymol blue indicator, 0.2 gram, was dissolved in 43 ml. of 0.01 *N* sodium hydroxide and diluted to 500 ml. with distilled water.

Potassium iodide–barium chloride solution, approximately 0.5 *N* with respect to each reagent.

Potassium iodate, C. P. grade.

Potassium permanganate, approximately 0.1 *N*.

Acetone, acid-free.

Apparatus

The apparatus is similar in design to one described in a previous article (*2*). Two changes were made in the reaction vessel: The bottom was enlarged to form a bulb and the cup on the side was constructed from a male standard taper joint. The bulb made it easier to dissolve many substances, while the joint afforded a better means of connecting the carbon dioxide–free air system to the apparatus and provided better protection against organic contamination. Since no carbon monoxide was formed during iodate oxidation, the slow combustion unit was deleted from the train. The modified apparatus is illustrated diagrammatically in Figure 1. For the protection and accurate measurement of the standard barium hydroxide solution the automatic pipet described by West (*4*) was employed.

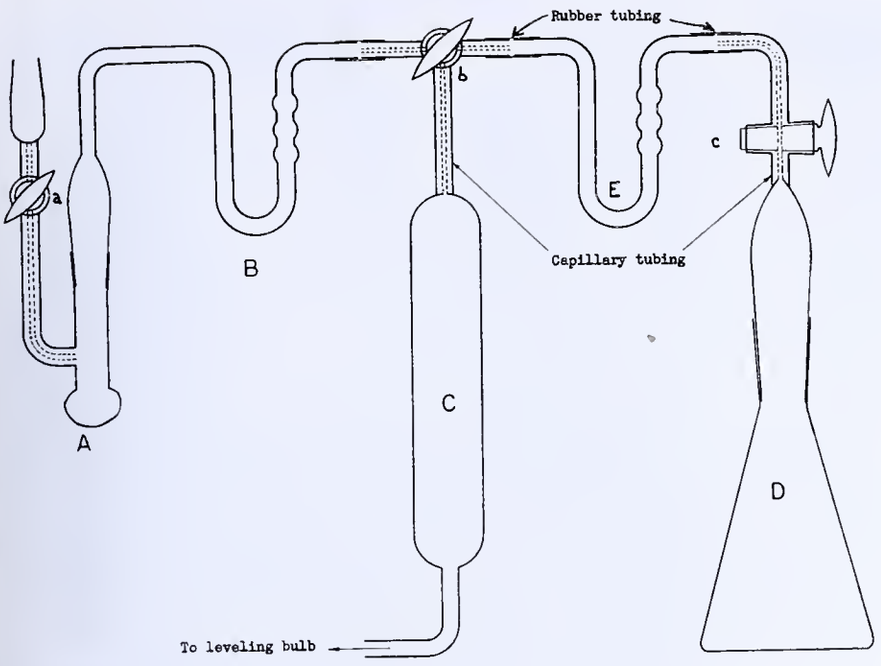

FIGURE 1. DIAGRAM OF MODIFIED APPARATUS

The heating bath was made from a 150-ml. beaker, wound with fifteen turns of No. 22 Nichrome wire. The temperature was controlled by a variable transformer (Adjustavolt, Standard Electrical Products Co., St. Paul, Minn.). Phosphoric acid was used as a bath medium. This arrangement permitted control of the temperature within ±5° C. with only an occasional adjustment.

Analytical Procedure

The absorption vessel, *D* (Figure 1), is evacuated by means of a water pump, and filled with air drawn through a soda-lime tower. It is then charged with 5 ml. of 0.1 *N* barium hydroxide accurately measured by an automatic pipet (*3*), and re-evacuated. The ground-glass joints are well lubricated with glycerol to prevent sticking. When charging the vessel with barium hydroxide care must be taken not to leave it open longer than necessary. One milliliter of the solution of potassium iodide–barium chloride is placed in U-tube *B* to remove iodine vapors. To ensure the removal of any hydriodic acid which may be formed, 1 ml. of 0.1 *N* potassium permanganate is placed in U-tube *E*.

Two to 5 mg. of organic matter and 50 to 70 mg. of potassium iodate are weighed in a glass boat and introduced into vessel *A* which is then connected (as shown in Figure 1) to the gas reservoir, *C*. Sufficient carbon-free sulfuric acid to cover the boat is introduced by means of stopcock *a*, which is then closed. At this time the charge in the boat, resting in the bulb of the reaction vessel, is agitated in order to dissolve the charge as much as possible before heating. The reaction vessel, even though connected to the system, has sufficient freedom of motion to permit considerable agitation. The bulb of the reaction vessel is now immersed directly in the heated bath.

The temperature of the reaction chamber is kept at approximately 210° ± 5° C. for the completion of the reaction by means of a phosphoric acid bath. This usually requires from 40 to 60 minutes. The gases which are generated during the reaction expand into gasholder *C*. After 20 minutes the Ascarite tower is connected to the apparatus through stopcock *a* and by operating stopcocks *a*, *b*, and *c* this gas is flushed into the absorption flask with approximately 50 ml. of carbon dioxide–free air. After 40 minutes, if the reaction is still incomplete (as evidenced by the continued evolution of small bubbles of gas) this flushing is repeated.

At the end of the heating operation stopcock *a* is again opened and 1 to 2 ml. of distilled water are introduced in order to decrease the free air space. The gas which has accumulated in *C* is transferred to *D*. Sufficient carbon dioxide–free air is then flushed through the system to sweep all the carbon dioxide into the evacuated flask, *D*, bringing it to atmospheric pressure.

The reaction vessel, *A*, is removed and its contents are transferred to a 500-ml. Erlenmeyer flask. While the reaction vessel is being dried in an oven, U-tube *B* is disconnected, cleaned, and recharged for the next run. It is important that the iodine be removed after each run, as the accumulation of too much iodine crystallizing in the male joint of *A* interferes with the recovery of carbon dioxide.

DETERMINATION OF CARBON DIOXIDE. *D* is now removed and allowed to stand for 20 minutes, 5 ml. of acid-free acetone (to improve the end point) are added, and the excess barium hydroxide is titrated to the thymol blue end point.

To prevent the diffusion of air into the flask during the titration, a rubber sheet (such as is used for a dental dam) is loosely fitted over the mouth of the vessel by means of a rubber band. The tip of the buret is inserted through a small hole in the rubber. The amount of carbon dioxide evolved is then determined from the amount of standard acid required to neutralize the excess base and that required in the case of the blank run.

DETERMINATION OF OXYGEN CONSUMED. The contents of reaction vessel *A* are diluted to approximately 100 ml. and boiled until the disappearance of the iodine color. The solution is then cooled, 1 gram of potassium iodide is introduced, and it is titrated with 0.1 *N* thiosulfate using a 10-ml. microburet. This measures the excess iodate.

VOLATILE COMPOUNDS. Compounds which are too volatile to weigh directly can be readily treated in the following manner. Fifteen to 20 grams of carbon-free sulfuric acid are weighed out in a dropping bottle provided with a ground-glass pipet. Approximately 100 mg. of the volatile compound are then mixed and dissolved in the acid and the solution is weighed, giving the organic content per unit weight. Portions are then removed from the dropping bottle for analysis. In addition to handling volatile compounds this procedure permits the microdetermination of carbon and hydrogen without the use of a microbalance.

BLANK DETERMINATION. It is important that the sulfuric acid be as free of organic matter as possible. Because the sulfuric acid may contain small amounts of oxidizable matter, it is necessary to run blanks on the acid in order to determine the amount of iodate (if any) consumed per milliliter of acid. This should be repeated with each new batch of acid.

In order to correct all possible sources of error (such as carbon dioxide from reagents, residual air, etc.) the measured charge of barium hydroxide (5 cc.) was compared to the standard acid after it had been subjected to a blank determination. The blank value for the barium hydroxide should also be redetermined with each new batch of acid. In series of blank runs the iodate consumption was 0.0 mg. per ml. of sulfuric acid. .Five milliliters of barium hydroxide required 9.10 ml. of hydrochloric acid by direct titration, while on blank runs only 9.04, 9.06, and 9.06 ml. were required.

CALCULATIONS. The milliliters of standard hydrochloric acid required for the blank determination, *A*, minus the milliliters of hydrochloric acid required to titrate the excess barium hydroxide, *B*, are equivalent to carbon dioxide evolved. Expressed as an equation

$$\frac{(A - B)\ (N \text{ of HCl}) \times 6 \times 100}{\text{mg. of sample}} = \% \text{ carbon in sample}$$

The per cent of hydrogen is calculated by means of Williams' equation (*5*)

$$\% \text{ H} = \frac{(\% O_R + 100 - 11/3\ \% C)}{8.93}$$

$$\% O_R = \frac{40}{214} \times \frac{(\text{mg. of } KIO_3 \text{ used})}{\text{mg. of sample}} \times 100 = \text{oxygen requirement in grams per 100 grams of sample}$$

TABLE I. COMPOUNDS OXIDIZED IN ONE HOUR OR LESS

Sample	Carbon Found	Carbon Theoretical	Hydrogen Found	Hydrogen Theoretical
	%	%	%	%
Benzoic acid	68.6	68.8	4.82	4.95
	69.1		4.81	
Vanillin	63.5	63.2	5.10	5.30
	63.4		5.26	
Sucrose	42.2	42.1	6.45	6.48
	42.1		6.43	
	42.3		6.38	
Hydroquinone	65.3	65.4	5.49	5.30
	65.3		5.29	
Succinic acid	40.7	40.7	5.05	5.12
	40.6		5.13	
	40.5		5.14	
Adipic acid	49.4	49.4	6.40	6.90
	49.4		6.78	
	49.3		6.78	
Sebacic acid	59.5	59.3	8.88	8.93
	59.5		...	
	59.6		8.87	
Anisic acid	62.7	63.1	5.34	5.30
	63.3		5.27	
	63.2		5.36	
Benzophenone	85.9	85.8	5.42	5.53
	85.7		5.48	
o-Iodobenzoic acid	33.9	33.9		
	34.1		...	
Cinnamic acid	72.5	72.9	5.69	5.44
	72.6		5.63	
Methanol[a]	38.0	37.6	12.5	12.5
	38.0		12.5	
	38.0		12.3	
Acetone	61.8	62.1	10.63	10.35
	62.1		10.5	
	61.8		10.2	
Ether	64.8	64.9	13.6	13.1
	64.7		13.8	
	64.3		13.8	
Acetophenone	80.3	80.0	6.67	6.77
	80.2		6.56	

[a] Contained 0.5 per cent of ethanol.

Results

The results using the micromethod are tabulated in Tables I and II. In Table I are listed the compounds which were completely oxidized in one hour or less.

In Table II are listed other compounds which were investigated. Some of these substances were completely oxidized but only after heating for a considerable length of time.

Discussion

The only advantage of the micromethod is its ability to handle very small samples. The modifications which were made in this method—namely, the method of determining the carbon dioxide and unreduced iodate—are just as adaptable to the semimicroprocedure.

Although numerous compounds are readily oxidized under these conditions it is evident from Tables I and II that the method does not have general applicability to all types of compounds.

On the basis of these studies it is the opinion of the authors that solubility and volatility are the dominant factors in a wet combustion. The effect of volatility is closely linked with the question of solubility. It is the soluble nonvolatile substances such as carbohydrate materials and aliphatic acids which are best adapted to wet-combustion procedures. However, highly volatile but soluble compounds such as ether, acetone, and methanol have been successfully treated by this procedure. This can probably be attributed to the formation of nonvolatile molecular compounds with the sulfuric acid.

Insoluble nonvolatile substances do not present much of a problem other than the question of time. Strebinger (*3*) reports the complete combustion with iodic acid of such insoluble materials as mineral oil. The ease with which these compounds are consumed depends for the most part on their resistance to oxidation. This may vary considerably between different compounds; *o*-iodobenzoic acid, for instance, is readily oxidized, but triphenylbenzene only after a considerable lapse of time.

Iodic acid in a more dilute sulfuric acid medium (40 per cent) has been shown to be specific in its behavior. Williams (*7*) has made a study of its specificity as an aid towards the determination of structure. Even in concentrated sulfuric acid medium this specificity still plays an important role.

The compounds which present the greatest difficulties are those which are relatively insoluble and tend to sublime easily. These materials are usually found among the aromatic compounds, particularly the halogen derivatives. Even such substances as benzoic and cinnamic acids may give considerable trouble if care is not taken to ensure their solution (as much as possible) before heating begins. In other cases, where the volatile compound is not so soluble, resistance to oxidation becomes an important factor and it then becomes a question of quantitative oxidation before some of the material escapes from the reaction mixture. This behavior is clearly demonstrated in some experiments with dibromodimethoxybenzene. In certain runs this compound was quantitatively oxidized. However, in some of the experiments the material would frequently sublime (in considerable amounts) out of the reaction mixture. Both experiments were carried out under like conditions.

From these studies it appears that any organic substance can be completely oxidizing, provided the material can be retained in the oxidizing medium for a sufficient length of time. The problem of successful analysis depends upon this point.

It is the opinion of the authors that wet combustion by iodic acid is best adapted to materials known to be readily oxidized by this reagent. For routine work with such materials or other compounds where combustibility by iodic acid can be previously tested (*6*), it offers a good tool for determination of carbon and hydrogen.

TABLE II. COMPOUNDS OXIDIZED AFTER ONE HOUR OR MORE

Sample	Time	Carbon Found	Carbon Theoretical	Hydrogen Found	Hydrogen Theoretical
	Hours	%	%	%	%
Phthalic acid	2	57.8	57.8	3.64	3.62
Butylphthalate	2	68.9	69.1	7.98	7.92
		69.2	..	7.95	..
Heptylphthalate	2	73.0	72.9	9.41	9.40
Methylcinnamate	2	74.2	74.1	6.05	6.18
Triphenylcarbinol	1	86.0	87.8	..	6.16
	4	87.8	..	6.04	..
	3	87.7	..	6.27	..
Triphenylbenzene	4	93.3	94.1	..	..
Dibromodimethoxybenzene	3	32.4	32.4	..	..
		32.2	..	..	..
		29.1	..	..	..
Anthraquinone	3	80.0	80.7	3.54	3.85
p-Phenyl phenacyl bromide	3	59.1	61.2	..	..
	1	58.7	..	..	..
Anthracene	2 (235°)	94.4	94.3	..	..
	2 (235°)	94.3	..	..	..
	1	89.7	..	..	..
Biphenyl	2 (185°)	62.8	93.5	..	..
	2	77.4	..	..	..
Naphthalene	2 (180°)	75.0	93.8	..	..
	1 (210°)	81.4	..	..	..

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Determination of Unsaturation in the Terpene Series

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The usual halogen absorption methods as well as those using standard potassium permanganate or standard perbenzoic acid were found unsatisfactory for the determination of unsaturation in terpenes. Quantitative hydrogenation using either a platinum or a palladium catalyst furnished satisfactory results with a variety of terpenes, but not with the resin acids. High-pressure reduction was also shown to be a suitable quantitative method for terpenes.

THE quantitative determination of unsaturation in the terpene series presents a rather different problem from that met with in determining unsaturation in most other classes of compounds. Because of the ease with which these compounds undergo substitution and isomerization, the usual halogen absorption methods are of very little use. Particularly with the Hanus method (*9*) it was noticed that the value obtained for α-pinene varied regularly with the size of the sample used. When the amount of reagent was kept constant, the smaller samples yielded higher values (Figure 1).

Other halogen absorption methods, some specifically claiming to eliminate substitution, such as a modified Hanus method using mercuric acetate (*8*), the pyridine–bromine–sulfuric acid reagent of Rosenmund and Kuhnhenn (*15*), and the Kaufmann method (*10*), were likewise found to be unsatisfactory. Similar observations have been recorded previously: Kubelka and Zuravlev found the iodine number of pinene and turpentine as determined by the Hanus and two other halogen absorption methods to be dependent upon the weight of sample (*13*) and the time of reaction (*14*); Kranz, Hrach, and Franta (*12*) reported that the iodine number of turpentine increased with increasing concentration of the iodine solution; Gal'pern and Vinogradova (*6*) obtained high values for pinene using the Kaufmann method; and Winkler (*20*) using a bromine–acetic acid solution found almost identical values for α-pinene and *l*-limonene which agreed with the theoretical value of neither.

Two methods not involving halogen absorption—namely, the use of a standard permanganate solution (*11*) and of a standard perbenzoic acid solution—were equally ineffective in solving the problem. The dependence of the double bond value of α- and β-pinene upon the ratio of the amount of reagent to the size of the sample taken, noted with the Hanus method, was again observed when perbenzoic acid was used (Figure 1), suggesting that the abnormal reaction occurred during the back-titration of the iodine liberated by the excess perbenzoic acid rather than during the original reaction of the perbenzoic acid with the terpene.

Quantitative hydrogenation has been used to determine unsaturation in several terpenes by Shaefer (*18*) using a palladium hydroxide catalyst, and a micromethod has been reported by Skarblom and Linder (*19*). The present authors found this general method, modified as described below, to be the most useful one investigated.

Shaefer (*18*) reported difficulty in effecting the addition of more than one mole of hydrogen to dipentene and Conant and Carlson (*4*) found the hydrogenation of this compound to stop after the absorption of about 1.6 moles. We found that dipentene recently fractionated through a good column readily absorbed the calculated amount of hydrogen, whereas low results were obtained with material purified merely by distillation over sodium.

Under the conditions used the method was not satisfactory for the quantitative determination of unsaturation in the

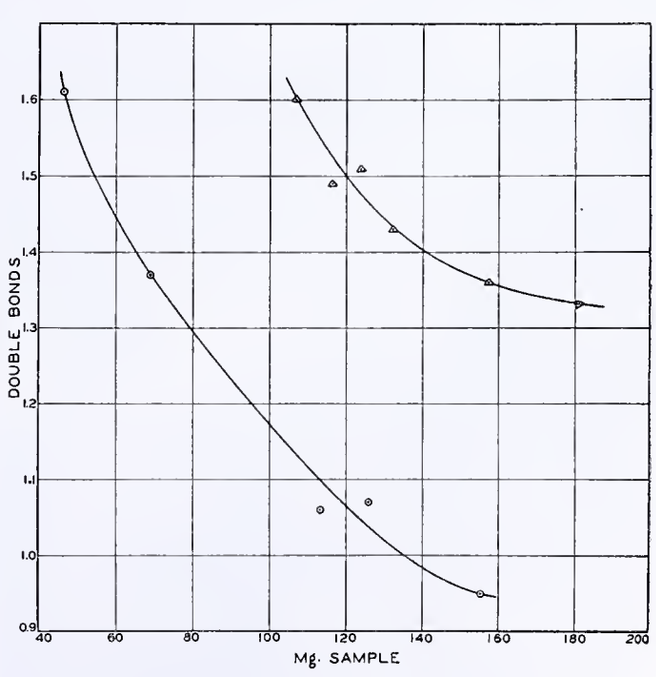

FIGURE 1. DOUBLE BOND VALUES FOR α-PINENE
⊙ Hanus method
△ Perbenzoic acid

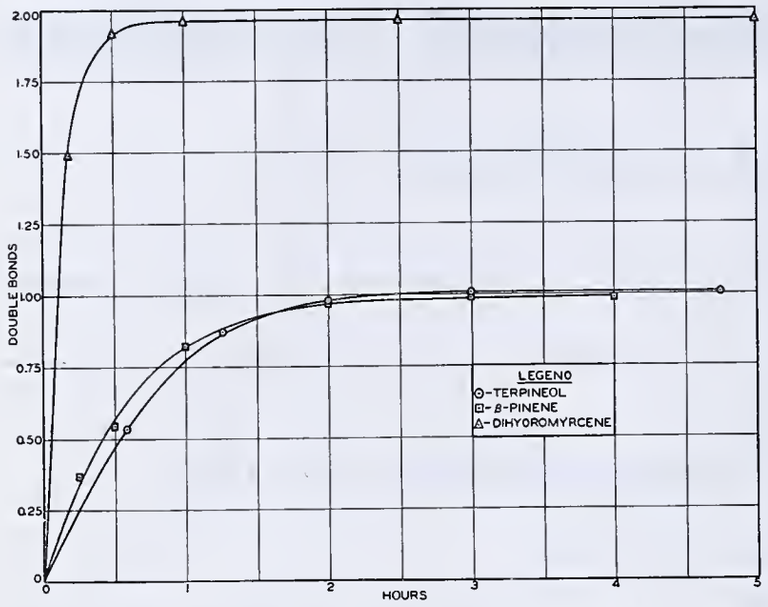

FIGURE 2. HYDROGENATIONS USING PALLADIUM–ZIRCONIUM OXIDE CATALYST

TABLE I. HYDROGENATION RESULTS

Compound[a]	Palladium–Zirconium Oxide: Double bonds	Palladium–Zirconium Oxide: Time, hours	Adams' Catalyst: Double bonds	Adams' Catalyst: Time, hours
α-Pinene (1)	0.99	3	1.03	0.5
β-Pinene (1)	1.00	3	0.99	0.25
Terpineol (1)	1.01	3	1.00	0.5
Pinocarveol (1)	0.94	1	0.99	0.25
Dipentene (2)	1.90	1.5	1.99	0.5
Dihydromyrcene (2)	1.96	1	1.95	0.3
Myrcene (3)	2.98	1	2.93	0.2
Alloöcimene (3)	2.91	1	2.90	0.2
Abietic acid (2)	1.67[b]	23	1.68[c]	23
l-Pimaric acid (2)	..	..	1.84[d]	47
Camphor (0)	0.00	1.5	0.02	3
Fenchone (0)	0.02	2	0.01	2
Borneol (0)	0.02	23	0.00	1
Fenchyl alcohol (0)	0.00	6	0.01	2
Dehydroabietic acid (one aromatic ring)	0.02	21	0.06	1.5

[a] Number in parentheses following name of compound indicates number of double bonds present.
[b] 100 mg. of catalyst used initially and 50 mg. more added after 3 hours. At this time 1.57 moles of hydrogen had already been absorbed.
[c] 50 mg. of catalyst used initially and 25 mg. more added after 2 hours. Hydrogen equivalent to 1.63 moles absorbed during first hour.
[d] Total of 200 mg. of catalyst added in 50-mg. portions used. 1.58 moles of hydrogen absorbed in first hour.

resin acids as exemplified by abietic and *l*-pimaric acids, since in these cases, despite the addition of fresh catalyst, the reduction became extremely slow after the initial rapid absorption of hydrogen corresponding to about 1.6 double bonds. It has been shown that it is possible to introduce two moles of hydrogen into these acids using elevated temperatures and with repeated reactivation of the catalyst (*16*, *17*).

Experiments with terpene alcohols and ketones possessing no double bonds showed that they were not attacked. In a like manner dehydroabietic acid, which contains an aromatic ring, remained unaffected under the conditions used.

Exploratory experiments showed that high-pressure hydrogenation using Raney nickel catalyst was also suitable for the determination of unsaturation in terpenes.

Experimental

All the compounds used were freshly distilled or freshly crystallized and had physical constants closely agreeing with the accepted values.

HYDROGENATION. The apparatus described by Fieser and Hershberg (*5*) was preferred to the many others described in the literature because of its simplicity of construction and ease of adaptation to the Burgess-Parr hydrogenation apparatus which is available in many laboratories and also because it eliminates rubber tubing in the absorption system. A 100-ml. buret was used instead of the 50-ml. buret mentioned by Fieser and Hershberg and a long-necked 50-ml. round-bottomed flask served as the reaction vessel. A series of experiments indicated that acetic acid (distilled over potassium permanganate) was a better solvent than ethyl alcohol or ethyl acetate. Both Adams' catalyst (*1*) and a palladium on zirconium oxide catalyst (10 per cent palladium) were found satisfactory. Adams' catalyst has the advantage of effecting the reduction in a much shorter time, whereas the palladium–zirconium oxide has the advantages of a smaller blank and availability by purchase.

Blanks were run using 10 ml. of acetic acid and given amounts of the catalysts and the corresponding blanks were subtracted from the gross volumes of hydrogen absorbed in the subsequent determinations. The size of sample chosen was such that 3 or 4 millimoles of hydrogen would be absorbed and 10 ml. of acetic acid and 25 mg. of Adams' catalyst or 50 mg. of the palladium–zirconium oxide catalyst were used in all cases, except as noted in Table I. (Later batches of the palladium catalyst purchased after the completion of this work were less active and the use of 100 mg. of catalyst was then advisable.) The times given for complete absorption can only be considered as approximate figures, and the shaking was of course always continued for some time longer to make certain that the absorption of hydrogen had definitely stopped. Some of the values obtained are given in

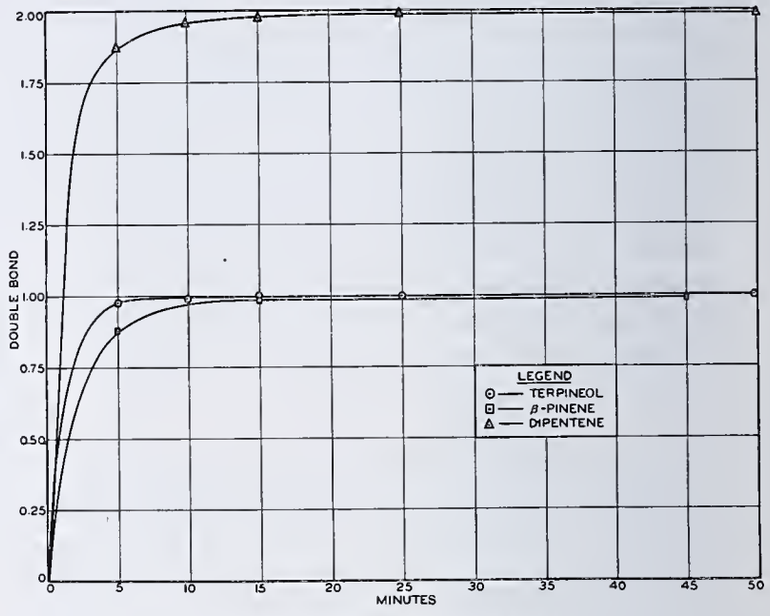

FIGURE 3. HYDROGENATIONS USING ADAMS' CATALYST

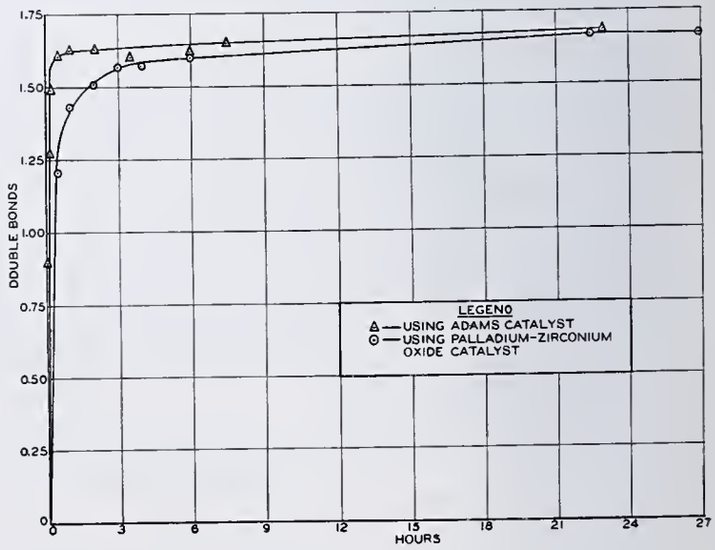

FIGURE 4. HYDROGENATION OF ABIETIC ACID

Table II for comparison with the results obtained by the other methods and a more complete list of results is compiled in Table I. Some typical rates of hydrogenation curves are shown in Figures 2, 3, and 4.

Perbenzoic Acid Titrations. Perbenzoic acid was readily and reproducibly prepared in good yield by the method of Braun (*3*) using benzoyl peroxide. One to 1.5 milliequivalents of the terpene were dissolved in 10.00 ml. of a dried, approximately 0.5 *N* solution of perbenzoic acid in chloroform and allowed to stand at 5° for 24 hours and then the excess perbenzoic acid was titrated in the usual manner (*3*). Blanks were simultaneously run on 10.00-ml. portions of the reagent. Other samples were allowed to stand at 5° from 48 to 96 hours, but the results showed clearly that in all cases the reaction was over at the end of 24 hours.

The other determinations, results of which are indicated in Table II, were carried out as described in the literature.

High-Pressure Hydrogenation. A bomb with a total void of 183 ml. was used, but the use of a glass liner (*7*) redueed the void to 142 ml. Calibration of this bomb and liner using acetone made up to a volume of 40 ml. with alcohol showed the pressure drop to be 3800 pounds per mole of hydrogen absorbed. Reduction of 17.09 grams of α-pinene made up to 40 ml. with alcohol was complete in about 12 hours at 75° (initial pressure 1670 pounds at room temperature). The pressure-drop was 480 pounds, which corresponds to 1.01 moles of hydrogen per mole of α-pinene. A similar experiment with β-pinene gave a value of 1.06 double bonds. Raney nickel catalyst (*2*) was used in all these experiments.

Table II. Comparison of Methods Investigated[a]

Method	α-Pinene (1)	β-Pinene (1)
Hanus	0.95–1.61[b]	1.12
Hanus + mercuric acetate	1.25	1.42
Rosenmund and Kuhnhenn	1.70	1.65
Kaufmann[c]	2.15	2.02
Potassium permanganate	0.90	1.23
Perbenzoic acid[d]	1.33–1.60[b]	1.37–1.63[b]
Hydrogenation (Pd)	0.99	1.00
Hydrogenation (Pt)	1.03	1.05

[a] Results are expressed as number of double bonds found per molecule, and the number in parentheses following name of terpene indicates number of bonds theoretically present.

[b] Depending upon size of sample. Other figures represent averages of check analyses.

[c] Kaufmann method gave a value of 2.15 double bonds for alloöcimene (3) and 1.99 for dipentene (2).

[d] Perbenzoic acid also gave following results: myrtenol (1), 0.89; pinocarveol (1), 1.23; myrcene (3), 2.17; alloöcimene (3), 2.35; dihydromyrcene (2), 2.10; dipentene (2), 2.03; terpineol (1), 0.87.

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Colorimetric Determination of Formaldehyde in the Presence of Other Aldehydes

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THE method of detecting formaldehyde in the presence of higher aldehydes suggested by Denigès (*3*) can be made semiquantitative in character with the aid of a colorimeter. The errors involved vary from 2 to 10 per cent, becoming greater as the proportions of higher aldehydes are increased. The limit of sensitivity under the experimental conditions described herewith is of the order of 0.02 mg. of formaldehyde in 5 ml. of solution. The test depends upon the fact that the magenta color given by Schiff's reagent with formaldehyde in the presence of sulfuric acid does not fade appreciably during 6 hours, whereas the color given by the higher straight-chain aldehydes, glyoxals, and their polymers fades completely within 2 hours. Trioxymethylene reacts the same as formaldehyde.

The reagent is prepared by first dissolving 0.5 gram of fuchsin in 500 ml. of water, then adding 5.15 grams of sodium bisulfite. Approximately 15 minutes later, 17 ml. of 6 *N* hydrochloric acid are added and the whole solution is allowed to stand for 3 hours. During this time the solution fades to a permanent, pale yellow color.

In a determination, 5 ml. of an aqueous solution of the substance to be analyzed are added to a mixture of 5 ml. of the Schiff's reagent and 1.2 ml. of 75 per cent sulfuric acid. A known comparison solution is made up at the same time using a standard formaldehyde solution and the two solutions are compared after they have stood for 2 hours in stoppered test tubes. Too long a time should not be allowed to elapse before the comparison is made, for even the color due to formaldehyde fades slightly on standing. Before results are considered final, the formaldehyde concentrations of the unknown and standard solutions should be within 5 per cent of each other. Accordingly, an ap-

Table I. Typical Analytical Data and Results

Compositions of Standard Comparison Solutions: Per cent H_2CO	Ratio of second aldehyde to H_2CO	H_2CO in Unknown: Present %	Determined %	Ratio of Second Aldehyde to H_2CO in Unknown	Error %
Formaldehyde Solutions					
0.0050	..	0.0067	0.0068	..	+1.5
0.0050	..	0.0050	0.0050	..	0.0
0.0040	..	0.0040	0.0039	..	−2.5
0.0015	..	0.0020	0.0019	..	−5.0
Formaldehyde-Acetaldehyde Solutions					
0.0040	50	0.0025	0.0024	40	−4.0
0.0040	50	0.0045	0.0048	20	+6.7
0.0040	5	0.0070	0.0068	3	−2.9
0.0040	10	0.021	0.020	10	−5.0
Formaldehyde-Propionaldehyde Solutions					
0.0040	25	0.0043	0.0040	16	−7.0
0.0040	25	0.0033	0.0031	20	−6.0

proximate calculation of the unknown is made first. If the two solutions differ by more than 5 per cent, one or the other is diluted sufficiently to satisfy this requirement and a new colorimetric comparison is made. Sometimes a third comparison is necessary. The concentration range for the most satisfactory colorimetric comparison is from 0.001 to 0.005 per cent formaldehyde.

Aqueous solutions of pure formaldehyde may be analyzed to a degree of accuracy dependent upon the colorimeter—i. e., within 2 or 3 per cent. However, other aldehydes tend to change the color and solutions containing appreciable quantities of other aldehydes may not be accurately compared with pure formaldehyde standards. In such cases the standard should be made up to contain the higher aldehydes in concentrations approximating those considered to be in the unknown. For example, in solutions containing acetaldehyde and formaldehyde, if the ratio of acetaldehyde to formaldehyde is between 10 and 100 the standard should be made up with a ratio near 50; if between 1 and 10 the standard ratio should be about 5; and if the ratio is less than 1 a pure formaldehyde standard may be used.

The method has been tested on mixtures of formaldehyde with acetaldehyde, propionaldehyde, glyoxal, methylglyoxal, biacetyl, and their polymers. It has been used satisfactorily in photochemical studies of acetaldehyde (*2*) and propionaldehyde. Table I contains a few typical analytical results obtained on unknown solutions.

In addition to the results described above, the Denigès qualitative method works very well in colorimetric capillaries (*1*). In such experiments the limit of sensitivity is about 0.02 microgram in 5 cu. mm. of solution.

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Determination of Sulfur in Organic Compounds

Oxidation of Sulfur of Cystine and Methionine, Combination of Parr Oxygen Bomb and Acidimetric Benzidine Method, and Determination of Small Amounts of Sulfur Compound Present as Contaminant in Organic Material

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IN CONNECTION with investigations concerned with the sulfur-containing amino acids and their derivatives, data pertaining to various analytical problems have accumulated in this laboratory. The material set forth below deals chiefly with the behavior of the sulfur present in the structure of cystine and methionine on oxidation by alkaline permanganate and by other wet-oxidation methods, the development of a practical analytical procedure in which combustion of the organic substance in the Parr oxygen bomb is followed by precipitation of sulfate as the benzidine salt and acidimetric determination of the latter, the effect of certain inorganic substances in this procedure and an investigation of its accuracy when applied to the determination of small amounts of organic sulfur in the presence of large amounts of sulfur-free material.

Alkaline Permanganate Method

The method as used by the authors is based on that reported by Blix (*2*), who showed that the sulfur of cystine was recovered with an accuracy of 98.9 ± 0.6 per cent when the substance was oxidized by an alkaline permanganate solution, the excess permanganate was reduced to manganous ion by hydrochloric acid, and the sulfate was determined as barium sulfate. The authors have compared this procedure with the alternate possibility of reducing the permanganate to manganese dioxide which is filtered off before precipitation of barium sulfate.

Twenty-five cubic centimeter portions of a solution of 1.6132 grams of l-cystine [which optical rotation (*18*) showed to be at least 99.7 per cent pure] and 50 millimoles of hydrochloric acid in a total volume of 250 cc. were analyzed as follows.

a. The sample of solution was heated for 2 hours on the steam bath with 30 cc. of 2 *M* sodium hydroxide and 1.50 grams of potassium permanganate (both of reagent grade) in a total volume of 150 cc. Excess permanganate was then decomposed by addition of 25 cc. of 7 *M* hydrochloric acid and gentle boiling. After partial neutralization (just acid to methyl orange) with sodium hydroxide, sulfate was precipitated by 35 cc. of a 0.05 *M* barium chloride solution and, after leaving on the warm steam bath overnight, filtered, ignited, and weighed.

b. After oxidation as under (*a*) a few cubic centimeters of methanol were carefully added to the hot solution, causing the formation of manganese dioxide and a colorless solution. After addition of 20 cc. of 2 *M* hydrochloric acid, which causes conversion of the manganese dioxide into a more easily filterable condition, the manganese dioxide was at once filtered and washed with hot water (containing about 1 per cent ammonium acetate to prevent colloidal passage of manganese dioxide) until a sample of the filtrate showed absence of chloride ion (about 8 washings). In the combined filtrate and washings sulfate was determined, after adjustment of the acidity, as under (*a*).

c. The determination was carried out as under (*b*) except that 4.5 grams of potassium permanganate were used. In the determination of the sulfate blank attributable to the potassium permanganate the procedure was similar to (*b*), except that cystine was omitted and 10.0 grams of potassium permanganate were used.

The results were as follows: sulfur found in potassium permanganate, 0.0176 and 0.0163 per cent. The sulfur content of the cystine was found, after deduction of the corresponding permanganate blanks, as 27.01 and 26.61 per cent according to procedure (*a*), 26.41 and 26.50 according to (*b*), and 26.51 and 26.71 according to (*c*). The theoretical value is 26.67 per cent. The mean value of procedures (*b*) and (*c*) corresponds to 99.5 ± 0.3 per cent of the theoretical, while procedure (*a*) gave 100.5 ± 0.8 per cent. In further work the procedure involving filtration of manganese dioxide was adopted because of its higher precision and accuracy, and because the color of the barium sulfate obtained in procedure (*a*) indicated the presence of manganese.

d. A semimicromodification (about 50 mg. of substance, 100 mg. of barium sulfate) which the authors have frequently used for the determination of sulfur in certain oxidation products of cystine (*15*) is as follows:

A 50-mg. sample of the substance, weighed into a 125-cc. Erlenmeyer flask to the nearest 0.05 mg., is dissolved in about 50 cc. of a solution containing 10 millimoles of sodium hydroxide and 0.50 gram of potassium permanganate. After heating the solution for 2 hours on the steam bath, 1.5 cc. of methanol are added, followed by 10 cc. of 2 *M* hydrochloric acid when reduction of the permanganate is completed. The precipitate is at once filtered and washed until free of chloride. In this case, where filtration is carried out at an acid reaction, addition of ammonium acetate to the wash water [see (*b*), above] proved to be unneces-

sary. The filtrates amounting to about 200 cc. are evaporated to about 100 cc. and, if the reaction is acid to methyl orange, precipitated with 15 cc. of 0.05 *M* barium chloride. The barium sulfate is isolated as usual and weighed with the same precision as the sample.

The percentage differences found between several duplicate determinations run in this way (*d*) on unknown substances were 0.04, 0.15, 0.59, and 0.08. A pair of determinations on l-cystine (the same material as above) gave 26.32 and 26.26, or a difference of 0.23 per cent and a mean value of 98.6 per cent of the theoretical. The mean precision in this modification is about 0.1 per cent, while the accuracy may be about 99 per cent.

The alkaline permanganate method has further given correct sulfur figures on cystine disulfoxide (*19*) and cysteine sulfinic acid (*6*), and it has been found convenient and productive of concordant results in the determination of the sulfur content of wool and of mixed copper salts obtained from wool hydrolyzates (*16*).

In the case of wool, 1-gram samples, 10 grams of permanganate, and 160 millimoles of sodium hydroxide in 150-cc. total volume were used, and 48 hours of heating on the steam bath were allowed to be certain of complete digestion of the wool. After filtration of the manganese dioxide and acidification some insoluble silica was filtered off before precipitation of barium sulfate. The results were 3.212 and 3.223 per cent of sulfur. In the case of the copper salts a 0.1- to 0.15-gram sample was dissolved in 20 cc. of 0.5 *M* hydrochloric acid and cupric ion was precipitated from the hot solution by hydrogen sulfide. The copper sulfide was washed with 0.005 *M* perchloric acid until free of chloride, the total filtrate (about 80 cc.) freed of hydrogen sulfide by passing a stream of carbon dioxide through it, and it was then subjected to alkaline permanganate oxidation in the manner outlined under (*b*). The differences within five pairs of determinations averaged 1.2 ± 0.7 per cent of the mean value of each pair.

Permanganate Oxidation of Methionine

An obvious advantage of the permanganate method is its direct applicability to dilute aqueous solutions. A sulfur determination method possessing this advantage would be of great value, for instance, in tracing the fate of the sulfur-containing amino acids in the fractionation of protein hydrolyzates by various chemical methods. It was important, therefore, to ascertain the response of methionine to permanganate oxidation, especially since under various other oxidative conditions methionine sulfur has revealed unusual resistance against complete degradation (*14*, p. 343). The results with permanganate were interesting. Two oxidations carried out according to method (*d*), except that all quantities were doubled, produced no measurable amount of barium sulfate. The following experiments make it probable that this result is caused by volatilization of sulfur-containing fragments (methyl sulfide, etc.).

One of the solutions to which barium chloride had been added was evaporated to dryness and redissolved, and a fraction of the solution was treated with aqua regia and magnesium chloride, as described in the next section. No evidence of the formation of sulfate by this oxidative treatment, which as shown below produces 80 to 90 per cent of the theoretical value when applied to methionine directly, could be obtained. The possibility of obtaining a different kind of oxidative cleavage of methionine by permanganate in acid solution was next examined. The procedure was as in the alkaline oxidation, except that 10 millimoles of hydrochloric acid instead of 20 millimoles of sodium hydroxide were used at the start. After heating on the steam bath, one sample for 0.75 hour and another for 1.5 hours, 15 cc. of 2 *M* sodium hydroxide were added and heating was continued for 2 hours, and after adding more permanganate (25 per cent of the original amount, since no unused excess was left) for a further 2 hours. The samples were then worked up as usual; however, no barium sulfate was obtained. Likewise, subsequent oxidation by aqua regia, in the manner applied following the direct alkaline oxidation, produced no sulfate whatever.

The quantitative results obtained by the alkaline permanganate oxidation on cystine and methionine (all of the sulfur and none of it, respectively, oxidized to sulfate) suggest the possibility of utilizing this behavior for the differential determination of methionine and cystine sulfur. If the observed distinction holds true when the compounds are present as parts of a protein structure, the difference between total sulfur and "permanganate sulfur" should correspond to methionine sulfur if no other sulfur is present. Preliminary experiments carried out on egg albumin, in which total sulfur was determined by the bomb method (see below) and the subfractions (inorganic, cystine, and methionine sulfur) according to Kassell and Brand (*5*), while at the same time oxidations with alkaline permanganate were run, showed the expected agreement between the methionine values within reasonable limits. However, no comprehensive comparisons have as yet been run to establish the general validity of the principle or its value compared with the differential oxidation method of Lugg (*8*).

Behavior of Methionine Sulfur in Wet-Oxidation Methods

The practical consequences of the difficulty of completely converting methionine sulfur to sulfate are obvious. Not only would availability of a reliable and simple method for the oxidation of methionine sulfur present in aqueous solution be of definite value in work concerned with the chemical isolation or the metabolism of this amino acid, but doubts about the adequacy of some of the customary methods may reflect more or less seriously, depending on the relative amounts of methionine involved, on the validity of many published analytical figures for the total sulfur content of proteins or of metabolic intake, intermediate, or output materials. The authors have, therefore, studied the response of methionine to some recently published oxidation procedures and some independent modifications.

A wet-oxidation method involving the protracted action, at slowly rising temperatures, of a mixture of nitric and perchloric acid and cupric salt, has been recommended for metabolic products by Pirie (*13*). Except for the statement that "80 to 90 per cent of the sulfur in *S*-benzylcysteine, or *S*-ethylcysteine added to urine, is converted into sulfate by this method", no data on the accuracy of the method are presented. The authors' results, given in Table I, show sulfur recoveries of 70 to 80 per cent in the case of methionine and, in addition, demonstrate that reasonable agreement between duplicates, especially when run simultaneously, cannot be taken as evidence of complete oxidation.

A seemingly simple and promising method is that of Zahnd and Clarke (*20*) in which oxidation is obtained by boiling with nitric acid and potassium chlorate (or nitrate), followed by fusion of the evaporation residue. Applicability is claimed to any type of organic substance except volatile sulfones or compounds yielding them, and good results are reported for various substances, including a close relative of methionine—*S*-ethylcysteine—on which consistent values of 97 per cent of the theoretical were obtained. However, as shown in Table I, application of the method to methionine yielded variable results below 90 per cent of the theoretical. A modified reagent, in which aqua regia is the oxidizing agent, gave likewise low results which regardless of variations of procedure fluctuated between 80 and 90 per cent. No better result was obtained when hydrobromic acid was substituted for hydrochloric acid, and when hydriodic acid was used instead the yield became much lower. The last experiment with hydriodic acid is of special interest because here the first stage of the treatment corresponds closely to the conditions under which, as was shown by Baernstein (*1*), methionine is nearly

Table I. Formation of Sulfate from Methionine by Methods of Wet Oxidation

Oxidation Method	Methionine Sample Substance	Solution	Sulfate Found
	Mg.	*Cc.*	*% methionine sulfur*
HNO_3, $HClO_4$, $Cu(NO_3)_2$; method of Pirie (*13*); initial reagent added: 5 cc. of Pirie's mixture	100.3	Aqueous, 10	80.4[a]
	100.3	10	81.4[a]
	100.3	10	78.7[b]
	100.3	10	77.8[b]
	95.8	0.2 *M* HCl, 10	69.6[b]
	47.4	5	79.1[b]
	9.5	1	78.2
	85.5[c]	None	78.4
HNO_3, $KClO_3$; method of Zahnd and Clarke (*20*); initial reagent added: 2.5 cc. of 10% $KClO_3$ and 1 cc. of concd. HNO_3	10.03	Aqueous, 1	89.8
	10.13	1	89.2
	10.13	1	83.9
Aqua regia, $MgCl_2$[d]	10.03	Aqueous, 1	90.3
	10.03	1	84.9
Aqua regia, $MgCl_2$, $HgCl_2$[e]	10.03	1	85.0
Aqua regia, $MgCl_2$, repeated treatment[f]	10.03	1	84.6
	10.03	1	87.2
Aqua regia, $MgCl_2$, delayed oxidation[g]	9.48	1	82.6
	9.48	1	91.6
HNO_3, HBr, $MgCO_3$[h]	9.48	1	88.7
HNO_3, HI, $MgCO_3$[i]	9.48	1	0.0
HI, followed by aqua regia, $MgCl_2$[j]	9.48	1	15

[a] Determined as $BaSO_4$; all others as benzidine sulfate by titration.
[b] 20-cc. portions of 250 cc. of solution used for titrimetric sulfate determination.
[c] Individually weighed sample; all others are aliquot volumes of solutions.
[d] 0.5 cc. of 0.34 *M* $MgCl_2$ in 4 *M* HCl, 3 cc. of concd. HCl, and 1 cc. of concd. HNO_3 were added to sample in large test tube. Solution, containing freshly heated quartz chip, was briskly boiled to dryness and residue was heated until no more nitric vapors were evolved. Sulfate was determined in aqueous or slightly acid (HCl) solution of residue.
[e] Procedure identical with [d] except that 0.2 cc. of 1 *M* $HgCl_2$, as a possible catalyst, was added to oxidizing mixture.
[f] After evaporation to dryness as under [d], 3 cc. of concd. HCl and 1 cc. of concd. HNO_3 were added, followed by [d].
[g] Treatment as under [d] except that sample with reagent mixture added was left in steam bath at 80° to 90° overnight before proceeding with evaporation.
[h] Sample, with 4.4 cc. of concd. HBr (d 1.38), 1 cc. of concd. HNO_3, and 60 mg. of $MgCO_3$ added, was left in warm steam bath overnight, evaporated to dryness, and after addition of 1 cc. of concd. HNO_3 again evaporated and heated. Residue was dissolved in minimum amount of concd. HCl and after addition of 20 cc. of H_2O benzidine sulfate was determined as usual.
[i] Procedure as under [h] except that instead of HBr 5.8 cc. of HI (d 1.70) were used.
[j] Sample was heated overnight in steam bath with 5.8 cc. of HI (d 1.70); then 3 cc. of 0.7 *M* $MgCl_2$ in 4 *M* HCl, 2 cc. of concd. HCl, and 2 cc. of concd. HNO_3 were carefully added, and evaporation to dryness was obtained by heating step by step (130–140°, 180–190°) in air bath, followed by evaporation, etc., with 3 cc. of concd. HCl and 1 cc. of concd. HNO_3, as under [d].

completely converted into homocysteine thiolactone. Apparently this compound on further degradation tends more toward the formation of volatile oxidation products than the unmodified methionine.

The general conclusion suggested by the results listed in Table I is that the oxidation of methionine in acid media is accompanied by the formation of volatile sulfur compounds. The extent to which sulfate is formed varies in individual experiments without any evidence of simple dependence on controllable working conditions. Occasional identical results in duplicate determinations are no evidence of completeness of oxidation to sulfate. Attention should be called to a recent publication by Masters (*9*) in which a wet-oxidation method is described which, according to the single determination on methionine reported, yields 101.4 per cent of the theoretical amount of sulfate with this compound. The method uses the same reagent as Pirie's (*13*, nitric acid, perchloric acid) except for the omission of copper salt, but the oxidative digestion is carried out much more slowly, being spread over approximately 30 hours with frequent additions of reagent.

Use of the Oxygen Bomb

In employing the oxygen bomb method the authors have based their technique on the procedure of Garelli and Saladini (*4*).

The combustion sample is weighed directly into a small silica crucible (4 cc.). Safe and complete combustion can be expected if the sample does not weigh more than 1 gram nor occupy more than one half of the crucible space. Depending on the sulfur content, the sample size has varied between 0.1 and 1 gram. The sample is thoroughly wetted with Decalin (decahydronaphthalene Eastman, practical, was found free of sulfur) using enough to bring the level of the liquid to the surface of the solid matter, without permitting the total charge of organic matter to exceed 2 grams; for about 0.1 gram of crystalline organic substances 0.5 cc. of Decalin was used as a rule. Finally 25 mg. of ammonium nitrate (reagent grade) are added and mixed in by means of a short piece of fuse wire which is left in the crucible. Ten cubic centimeters of water are put in the bottom of the bomb (Parr oxygen bomb) the crucible is wired (*12*) and placed in the bomb, and the bomb is carefully closed. It is filled with oxygen to a pressure of 30 atmospheres, placed under water in a pail, and if no gas leakage is evident, connected with a 110-volt circuit. The circuit contains, in addition to a knife switch, a resistance board with three lamp sockets, which are connected in parallel. A 25-watt bulb is inserted and if the switch is momentarily closed completeness of the circuit is established by the glow of the lamp filament. The charge is best ignited by placing two 200-watt bulbs in the circuit and closing it for a second—i. e., by using a 4-ampere current. Although under these conditions there is no visible glow of the filament (or cessation to indicate occurrence of the ignition), the use of the invisible but stronger current is preferable to the visible one obtained with bulbs of higher resistance (lower wattage). In certain special cases (substances containing inert inorganic matter or material otherwise difficult to ignite), a low-amperage current will not induce a full explosion in the bomb but merely a slow combustion, indicated by duration of the glow of the lamp filament for as long as one minute, and usually resulting in incomplete oxidation. If ignition has taken place, the bomb is warm to the touch.

Although the authors have used the bomb with complete safety for over 6 years, the electric controls are located at a safe distance, as a matter of precaution. At least one hour is permitted to elapse before the bomb is removed from the pail. It is then rinsed from the outside and the gases are slowly released by means of the valve control. The 1-hour waiting period is essential to permit completion of the slow reaction $SO_3 + H_2O \rightarrow H_2SO_4$, as was demonstrated by Garelli and Saladini (*4*). The authors' evidence confirms this: cystine (sulfur calculated, 26.67 per cent) gave 26.34 and 26.18 per cent of sulfur when the bomb was opened after 10 minutes, while with a 1-hour waiting period 26.57, 26.60, and 26.68 per cent were obtained, or 99.8 ± 0.2 per cent of the theoretical, while optical rotation (*18*) indicated a purity of 99.7 ± 0.2 per cent

After opening the bomb, the valve in the cover and the bomb interior are carefully washed out with water which contains 8 drops of methyl orange (0.1 per cent aqueous solution) per liter, until all traces of acid reaction have been removed. To the combined washings 1 cc. of concentrated ammonia is added and the solution is heated. A small amount of iron hydroxide, together with any insoluble particles, is then removed by filtration, and after proper acidification the solution is ready for the determination of sulfur, either as barium sulfate or volumetrically as benzidine sulfate. If the latter method is to be employed, the bomb washings are ordinarily made to 200 or 250 cc. and determinations, in the manner described in the following section, are run on 20-cc. portions. When the amount of sulfate present is small, 40-cc. portions may be used, or all of the bomb washings may be used after evaporation to about 20 cc.

Acidimetric Benzidine Method

The authors' procedure is based on the method of Fiske (*3*) in which acetone is employed to reduce the solubility of the benzidine sulfate and to facilitate filtration and washing. The acidimetric determination of benzidine sulfate is much better suited for microanalytical quantities than for macro amounts, because of the sluggishness with which benzidine sulfate goes into solution on titration with alkali, even in the heat. The authors have found 20 cc. of an approximately 0.003 *M* sulfate solution, corresponding to about 2 mg. of sulfur, a convenient quantity for an accurate determination, when titrating with 0.05 *N* sodium hydroxide from a 5-cc. microburet graduated to 0.01-cc. (equal to about 1-mm.) intervals. The buret is calibrated and the alkali is standardized against potassium acid phthalate with phenolphthalein as indicator.

For the precipitating reagent purified benzidine hydrochloride is prepared from benzidine (reagent grade) according to

the method of Letonoff and Reinhold (*7*), and a solution of 0.086 *M* benzidine in 0.23 *M* hydrochloric acid is prepared.

To 20 cc. of the sulfate solution in a 100-cc. beaker, 2 drops of bromophenol blue (0.1 per cent) are added and the solution is adjusted to a slight acidity (indicator just yellow, *11*) by approximately *N* hydrochloric acid or ammonia. Then 4 cc. of benzidine reagent and, after 2 minutes, 8 cc. of 95 per cent acetone are added. After 15 minutes the precipitate is filtered on the pad of a Fiske filtration tube (*3*) which is permanently lodged in a clean rubber stopper that fits a suction flask. It is desirable to have several of these units. The hole in the authors' tubes has a diameter of 2 or 3 mm., and it is convenient to form a thin but firm filter pad by using a disk of 10-mm. diameter of fluffy blotterlike filter paper (oil filter, Durieux No. 127) which is sealed at the edges by a small amount of an aqueous paper pulp suspension. The beaker and the interior of the filter tube are carefully washed, successively with three 2-cc. and one 10-cc. portion of 95 per cent acetone.

The filter tube is now placed over a 125-cc. Erlenmeyer flask (the rubber stopper sits on the rim of the flask) and precipitate and pad are poked through the hole into the flask. For this operation a platinum rod 10 cm. long and 2.5 mm. in diameter is used, tapered at one end over a length of 1 cm. to a blunt tip. The use of copper wire instead of platinum led to erroneous results (see below). Rod and tube are rinsed with a little water, the rod is returned to the beaker, and the flask, with the tube remaining on it, is heated on a hot plate until the contents boil. After addition of 2 drops of phenol red (0.1 per cent), approximately one half of the amount of sodium hydroxide which is estimated to be required is run from the buret through the tube into the flask, followed by rinsing with about 5 cc. of water. After about 0.5 minute of gentle boiling the inside of the tube and the outside below the stopper are carefully rinsed with about 10 cc. of water from the wash bottle and the tube is removed and placed in the beaker. The total volume should now be approximately 20 cc. Titration is then continued with intermittent boiling until no visible amounts of undissolved benzidine sulfate are left and the solution retains a pink shade on boiling. At this point the hot solution is carefully poured back into the original beaker, passing through the filter tube, and after thorough swirling and complete dispersion of any remaining paper wad, returned to the flask. The titration is then continued to a definite pink which must persist on continued boiling.

The principles of this procedure were originally proposed by Fiske (*3*) for metabolic sulfur determinations and the limits of precision and accuracy were not examined, except that in the twelve cited comparisons between titrimetric and gravimetric determination the titrimetric values averaged 100.7 ± 0.9 per cent (average error) of the gravimetric ones. In order to obtain information on these points the authors have, in a number of actual analyses in which the organic substance was burned in the oxygen bomb, performed the sulfate determination by both the Fiske procedure and the conventional barium sulfate method, using separate portions of the bomb washings. The results of these determinations are listed in Table II. The mean difference between the results of the two procedures is −0.4 ± 0.7 per cent.

In 82 cases where duplicate determinations by the benzidine method have been carried out on portions of the same solution, the average difference was 0.009 cc. (±0.006, average deviation). The lowest difference was 0.000 (8 times), the most frequent 0.002 (15 times), and the highest 0.026 (2 times); 0.009 cc. of 0.05 *N* sodium hydroxide corresponds to 0.007 mg. of sulfur or 0.05 mg. of barium sulfate. It therefore seems that under favorable circumstances (dependable buret calibration, alkali standardization, practiced operator, etc.) the benzidine results are at least the equals of barium sulfate results in accuracy and precision. The benzidine method is superior in that it is much less time-consuming and requires only a fraction of the material needed for the barium sulfate method.

Effect of Inorganic Substances in Oxygen Bomb Combustion and Benzidine Sulfate Titration

It has long been recognized (*11*) that the presence of excessive amounts of various ions, such as chloride or phosphate, can be the cause of disturbances in the precipitation of benzidine sulfate. Since in the combustion products of ordinary organic substances such salts are not present, the benzidine method seems eminently suited as an adjunct to the oxygen bomb method of combustion. When inorganic matter is present in the substance to be analyzed the applicability of the combination of the two methods may be expected to encounter limits which should depend on the one hand on the effect of the inorganic component on the combustion process in the bomb, and on its interference in the precipitation or titration of benzidine sulfate on the other. Some information on these points has come to light in the course of sulfur determinations which the authors have run on certain crude mixtures of amino acids and sodium chloride and on combinations of mercury salts and amino acids.

Disturbances were encountered when a rod of copper instead of platinum was used in the handling of the benzidine sulfate. In the course of sulfur determinations the authors used a stout copper wire for a considerable time. During this period some entirely satisfactory results were interspersed with a number of cases in which multiple determinations by the benzidine method differed by as much as 11 per cent, and others in which the benzidine result was as much as 14 per cent lower than the barium sulfate result. Since these difficulties ceased entirely as soon as platinum was substituted, some interaction of the acid precipitate with copper must be suspected as their cause.

Turning to the effect of sodium chloride, a 1-gram sample of a crude amino acid precipitate which contained 50 per cent of sodium chloride was successfully ignited in the usual manner, leaving a slightly colored residue in the crucible. Two benzidine sulfate determinations on fractions of the solution gave 1.020 and 1.041 per cent of sulfur. Another ignition, of a 500-mg. sample, gave 1.036 and 1.040 per cent of sulfur. A different product of similar nature, containing 45 per cent of sodium chloride, yielded by the benzidine method 1.28 and 1.23 per cent of sulfur on two separate ignitions of 343 and 251 mg., respectively. Fiske

Table II. Comparisons between Gravimetric and Volumetric Results of Sulfate Determination

Sample, Substance		$BaSO_4$ Method		Benzidine Method		Difference between Benzidine and $BaSO_4$ Results
	$BaSO_4$	S	NaOH	S	
	Mg.	*Mg.*	%	*Cc.*	%	% *of $BaSO_4$ result*
l-Cystine (S calcd., 26.67%)	201.8[a]	155.6	26.46	1.276	26.38	−0.75
		157.1	26.71	2.506	26.40	
S-(*p*-tolylmercapto)-cysteine[b]	105.2[c]	163.5	26.68	2.585	26.01	−2.50
	101.1[c]	155.3	26.37	2.493	26.10	−1.02
Mixture 3B[d]	895.2[c]	109.9	2.108	1.800	2.093	−0.75
Mixture 3A[d]	392.9[e]	56.2	2.456	0.327	2.475	+0.77
Mixture 4[d]	662.8[c]	163.9	4.246	2.682	4.212	−0.80
Impure *l*-metbionine 4	97.9[c]	80.4	14.10	1.336	14.20	+0.71
Impure *l*-metbionine 2A	101.3[c]	108.9	18.46	1.802	18.51	+0.27
Impure *l*-methionine 2B	99.8[c]	121.1	20.83	...	...	...
	109.6[c]	...	...	1.640	20.74	...
				1.648	20.84	−0.19
l-Methionine (S calcd., 21.49%)	99.8	125.2	21.50	2.060	21.48	−0.09

[a] Combined washings of two bomb combustions of about 100 mg. each made to 500 cc. and weighed. Weighed 200-cc. portions used for $BaSO_4$ method, and weighed 10- and 20-cc. samples for benzidine method.
[b] Prepared by T. F. Lavine (unpublished).
[c] Bomb washings made to 250 cc.; 200 cc. used for $BaSO_4$ precipitation and 20 cc. for benzidine method.
[d] Metbionine-containing amino acid mixture from bydrolysis of egg albumin.
[e] Same as [c] except that only 7 cc. used for benzidine procedure.

Table III. Determinations of Sulfur in Presence of Mercury

Substance or Mixture	Weight of Sample Ignited	Bomb Washings Treated with Ammonia	Fraction of Bomb Washings Used for Benzidine Sulfate Determination	Sulfur Found	
	Mg.		%	*Wt. %*	*% of S present*
5H 2[a]	262.8	Yes	40	0.264	..
	289.2	Yes	40	0.260	..
	310.0	No	16	0.573	..
5H 5[a]	299.2	Yes	40	1.82	..
	658.3	Yes	40	1.40	..
l-Methionine + $(CH_3COO)_2Hg$	696[b]	Yes	8	14.48[c]	71
l-Methionine + $HgCl_2$ + $(CH_3COO)_2Hg$	582[d]	Yes	8	15.07[c]	74
	698[e]	No	8	21.45[c]	103
Benzoic acid + $HgCl_2$ + $(CH_3COO)_2Hg$	699[f]	No	16	0.00	..
l-Methionine mercury compound[g]	318.3	No	8	4.18	103
	357.7	No	8	4.20	104

[a] Methionine-containing precipitate obtained from protein hydrolyzate by mercuric chloride and mercuric acetate.
[b] Mixture of 115.0 mg. of *l*-methionine and 581 mg. of mercuric acetate.
[c] Calculated for methionine added; latter, isolated from egg albumin, gave on separate determinations 20.74, 20.84% S (Table II), indicating purity of 97 per cent.
[d] Mixture of 108.9 mg. of *l*-methionine, 365 mg. of mercuric chloride, and 108 mg. of mercuric acetate, approximating molar ratio 2:4:1 of methionine mercury compound (*17*).
[e] Mixture of 122.3 mg. of *l*-methionine, 445 mg. of mercuric chloride, and 131 mg. of mercuric acetate, approximating molar ratio 2:4:1 of methionine mercury compound (*17*).
[f] Mixture of 123 mg. of benzoic acid, 445 mg. of mercuric chloride, and 131 mg. of mercuric acetate, run as control for possible effect of mercury compounds.
[g] $(CH_3—S—CH_2—CH_2—CH(NH_2)—COO)_2Hg + 4HgCl_2$ (*17*, p. 368).

obtained good results by his method when the ratio of chloride (as sodium chloride) to sulfur was as high as 60, and the authors' results, in which that ratio is about 30, conform with his. Absence of carbonized residues in the bomb and the agreement between samples of different size further indicate that satisfactory results may be expected from oxygen bomb combustion in the presence of considerable inorganic matter.

A special situation was encountered in the presence of mercury salts. In connection with work on the isolation of methionine from protein hydrolyzates by means of mercury salts (*14*, *17*) determination of the sulfur content of a number of crude precipitates containing 60 to 65 per cent of mercury and about 15 per cent of chlorine became necessary. No difficulty was encountered in obtaining complete combustion in the bomb of samples as large as 650 mg. A considerable amount of ash, consisting largely of mercury and mercurous chloride was, of course, found in the bomb after ignition. Addition of excess ammonia to the bomb washings led to the precipitation of additional black mercury salts. Precipitation of benzidine sulfate in the filtrate and its titration offered nothing unusual. However, while duplicate ignitions resulted in substantially identical values when the size of the sample was similar, a decrease of 23 per cent in the percentage of sulfur found was encountered when the size of the sample was doubled. Investigation showed that in the presence of mercury salts a large fraction of the sulfate escapes determination by being incorporated in the ammonia precipitate, probably in the form of basic salts, thus producing results 25 to 55 per cent too low. However, when the treatment of the bomb washings with ammonia was omitted, and only the insoluble residue was filtered off, consistent results were obtained which exceed the theoretical value by about 3 per cent. The reason for this inaccuracy has not been ascertained. As is indicated in Table III, no evidence was found of a blank contribution of the mercury salts. However, results within 3 per cent of the actual value are satisfactory for many purposes, and the oxygen bomb–benzidine titration method can in such cases be recommended, on account of its simplicity, for the determination of sulfur in the presence of mercury.

Application of Bomb-Benzidine Combination to Determination of Methionine in Leucine

The difficulty of completely removing methionine from leucine specimens of protein origin is well known (*10*). The

Table IV. Determination of Small Amounts of Sulfate and Sulfur

Compound	H_2SO_4, 0.000253 *M*	H_2O	Time for Precipitation	0.04544 *N* NaOH		
				Used	Corrected	Calculated
	Cc.	*Cc.*	*Min.*	*Cc.*	*Cc.*	*Cc.*
Sulfate determination only	0.00	80	15	0.004		..
	0.00	80	15	0.000	0.001 ±	..
	0.00	80	15	0.000	0.001	..
			Hours, 0° C.			
	0.00	80	18	0.024		..
	0.00	80	18	0.018	0.021 ±	..
	0.00	80	18	0.022	0.002	..
	0.00	20	18	0.030		..
	0.00	20	18	0.026	0.022 ±	..
	0.00	20	18	0.010	0.008	..
	5.00	15	18	0.083		..
	5.00	15	18	0.097	0.064 ±	0.056
	5.00	15	18	0.079	0.008	..
	10.00	10	18	0.136		..
	10.00	10	18	0.126	0.108 ±	0.111
	10.00	10	18	0.128	0.008	..
Bomb combustion and sulfate determination						
Reagent blank[a]	...	200	18	0.165	...	..
	...	200	18	0.155	...	..
dl-Leucine, 698.8 mg.	...	200	18	0.157	...	..
dl-Leucine, 803.7 mg.	...	200	18	0.165	...	..
dl-Leucine, 701.4 mg. + *dl*-methionine, 0.959 mg.[b]		200	18	0.423	0.431 ±	0.444[c]
dl-Leucine, 702.3 mg. + *dl*-methionine, 0.959 mg.[b]		200	18	0.439	0.008	..
Purified *l*-leucine, 499.0 mg.	...	200	18	0.284	...	..
Purified *l*-leucine, 481.7 mg.	...	200	18	0.270	...	..

[a] In these and following determinations bomb ignition was performed using 1.0 cc. of Decalin and 25.0 ± 0.5 mg. o ammonium nitrate.
[b] Methionine and leucine mixed as described in text.
[c] Average of the two reagent blanks and the two *dl*-leucine runs served as blank value.

authors have applied the combination of oxygen bomb combustion and benzidine sulfate titration to the problem of determining the residual methionine present in a highly purified sample of natural leucine.

The procedure was as described above, with a few modifications. The bomb washings were evaporated to dryness on the steam bath after they had been filtered following the addition of ammonia. The evaporation residue was then redissolved in 20 cc. of water and benzidine sulfate was precipitated after proper acidification. Further, in order to ensure completeness of crystallization of benzidine sulfate even near its solubility borderline the precipitate was filtered only after it had stood overnight at about 0° C. Finally, each determination was accompanied by an exactly corresponding blank determination.

Table IV shows that with these precautions small amounts of sulfate are determined with an accuracy equal to the analytical precision and that amounts of the order of magnitude of the reagent blank may escape determination when insufficient time is permitted for crystallization.

Finally, complete determinations were run on the reagents only, as well as on a sample of synthetic leucine (*dl*-). The identical values of the two pairs of determination show that the synthetic product is, as would be expected, free of sulfur. The magnitude of the blank values is noteworthy. Systematic experiments in which one component was varied at a time showed that the various components are responsible approximately as follows: 1 cc. of Decalin, 0.00 cc. (0.05 *N* sodium hydroxide); 25 mg. of ammonium nitrate, 0.12 cc.; 200 cc. of water, 0.02 cc.; and 4 cc. of benzidine hydrochloride solution, 0.01 cc. Careful control of the amount of ammonium nitrate added as oxidation catalyst and use of the purest grade available are advisable because of its large contribution to the blank value and because erratic values and evidence of incomplete combustion were encountered when excessive amounts (more than 100 mg.) were used. The role of the blank value in the benzidine determinations of the preceding sections is greatly diminished by the fact that not more than 8 per cent of the total bomb washings were employed there.

For the next pair of determinations synthetic mixtures of *dl*-leucine and *dl*-methionine were prepared by adding 0.400-cc. portions of a solution of 120 mg. of *dl*-methionine in a volume of 50 cc. to quartz crucibles containing the 700-mg. samples of *dl*-leucine; after mixing with a piece of fuse wire the crucible and contents were dried overnight at 100°. The results show that the added methionine sulfur, corresponding to a 0.03 per cent sulfur contamination, is recovered with an accuracy of about 96 ± 3 per cent. The final pair of determinations shows the results obtained on the natural leucine. Calculation yields the figures 0.0180 and 0.0166 per cent for the sulfur content. A conservative evaluation of these results and the control and blank determinations seems to justify the statement that the leucine in question contains 0.017 ± 0.002 per cent of sulfur.

Summary

A convenient procedure for the oxidation of organic sulfur compounds, preparatory to sulfur determination, by alkaline permanganate is described. No sulfate is formed from methionine by this procedure. Incomplete and variable oxidation of methionine sulfur is shown to occur in some other wet-oxidation methods. A tested procedure for the determination of sulfur in organic compounds is described in which the substance is burned in a bomb by compressed oxygen and the sulfate formed is determined acidimetrically as benzidine sulfate. The presence of sodium chloride and mercury salts is shown within certain limits not to interfere in this method. Its successful application to the accurate determination of a few hundredths of 1 per cent of sulfur present in an organic substance is described.

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Simple Condenser for Use during Digestion Operations

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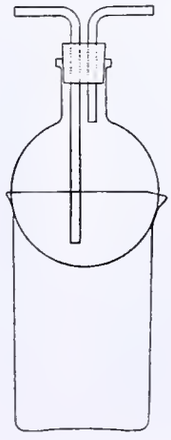
When it is necessary to employ beakers for longtime digestions, a simple condenser of the type described below presents several advantages over the watch glass conventionally used as a cover. In the first place, evaporation is practically eliminated—a rather important factor when expensive reagents, such as 30 per cent hydrogen peroxide, are involved. Furthermore, where a mixture of reagents is used, their initial ratio is well maintained despite any wide difference which may exist between their boiling points. One who has had occasion to digest solids in a mixture of nitric and sulfuric acids for extended periods will appreciate the difficulty in keeping reasonably constant the composition of such a mixture.



The condenser in question is nothing more than a round-bottomed, short-necked, Pyrex flask carrying a two-hole rubber stopper fitted with two pieces of glass tubing bent at right angles. One serves as an intake and the other as an outlet for water. It is desirable that the intake tube be extended to a point close to the bottom of the flask. This improvised condenser may be of any convenient diameter, dependent upon the size of the beaker with which it is to be employed.

The apparatus depicted may, of course, be employed as a single unit or in series.

Determination of Sulfate in the Presence of Chromate

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IN A STUDY of the system sodium sulfate–sodium chromate–water (*1*) it was found necessary to analyze solutions and solid mixtures containing both sodium sulfate and sodium chromate. Accurate analysis of sulfate-chromate mixtures for sulfate is made difficult by contamination of the barium sulfate precipitate with coprecipitated barium chromate, which forms mixed crystals with barium sulfate. To prevent this coprecipitation, the chromate may be reduced to chromic salt, acetic acid added, and the sulfate precipitated in the usual way. Willard and Schneidewind (*3*) describe and discuss this method in some detail.

In this paper, an alternative method for the determination of sulfate in the presence of chromate is described. Sulfate is precipitated from acid solution with barium chloride in the usual manner, and the necessary correction for coprecipitated barium chromate is determined and applied.

Reagents and Solutions

SODIUM SULFATE. Reagent grade sodium sulfate was twice recrystallized as decahydrate, the crystals being separated centrifugally from the mother liquor. They were then placed in a platinum dish and dehydrated by heating, gently at first, then strongly in a muffle furnace. The mass was powdered in a mortar, heated again, and kept in a weighing bottle in a desiccator until used.

SODIUM CHROMATE. Pure sodium chromate was prepared from reagent grade sodium dichromate by the method of Richards and Kelley (*2*), which differs from the more usual methods of purification in that it removes sulfate, the sodium salt of which is isomorphous with sodium chromate in all its hydrated forms (*1*). The salt thus prepared was dehydrated and kept in a weighing bottle in a desiccator.

SODIUM CARBONATE. Reagent grade anhydrous salt was used without further purification.

BARIUM CHLORIDE SOLUTION. Reagent grade salt, without further purification, was dissolved in distilled water.

SODIUM THIOSULFATE SOLUTION, 0.05 *N*. About 32 grams of reagent grade salt were dissolved in 2.5 liters of freshly boiled distilled water in a bottle which had been thoroughly steamed. This solution was standardized against twice recrystallized reagent grade potassium dichromate.

IODINE SOLUTION. About 16 grams of iodine were dissolved in 2.5 liters of potassium iodide solution containing about 20 grams of potassium iodide per liter.

STARCH SOLUTION. This solution was freshly prepared from potato starch each day on which it was needed.

TABLE I. REPRESENTATIVE RESULTS

Na_2SO_4 Taken	Na_2CrO_4 Taken	Wt. of Ppt.	Na_2SO_4 (Uncorrected)	$BaCrO_4$ in Ppt.	Na_2SO_4 Found	Error
Gram	*Grams*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
0.0162	1.93	0.0424	0.0258	0.0162	0.0159	−0.0003
0.0291	2.18	0.0646	0.0393	0.0159	0.0294	0.0003
0.0468	0.345	0.0810	0.0493	0.0045	0.0466	−0.0002
0.0550	0.340	0.0935	0.0569	0.0031	0.0550	0.0000
0.0596	0.155	0.0992	0.0604	0.0012	0.0596	0.0000
0.0608	0.124	0.1023	0.0623	0.0017	0.0612	0.0004
0.0760	0.339	0.1297	0.0789	0.0039	0.0766	0.0006
0.0811	0.11	0.1370	0.0834	0.0032	0.0814	0.0003
0.0827	0.166	0.1373	0.0836	0.0018	0.0825	−0.0002
0.0850	0.328	0.1475	0.0898	0.0078	0.0850	0.0000
0.0935	0.268	0.1581	0.0962	0.0058	0.0927	−0.0008
0.1000	0.102	0.1665	0.1013	0.0009	0.1008	0.0008
0.1127	0.802	0.2017	0.1227	0.0179	0.1119	−0.0008
0.1153	0.758	0.2080	0.1266	0.0178	0.1157	0.0004
0.1490	1.020	0.2581	0.1571	0.0135	0.1489	0.0001
0.1503	0.093	0.2485	0.1512	0.0015	0.1503	0.0000
0.1868	0.398	0.3098	0.1885	0.0020	0.1873	0.0005
0.2213	0.285	0.3770	0.2295	0.0126	0.2218	0.0005
0.2482	0.169	0.4093	0.2491	0.0005	0.2488	0.0006

Procedure

Dissolve the sample in about 75 ml. of water and add 5 ml. of concentrated hydrochloric acid. Heat to boiling, and add very slowly, dropwise, from a separatory funnel, about 75 ml. of barium chloride solution of such concentration as to give a small excess of barium ion over that needed to precipitate all the sulfate. After adding the precipitant, digest the mixture just below the boiling point for at least 3 hours. Cool, filter off the precipitate on a quantitative paper, and wash thoroughly to remove all traces of chromate from the paper. The chromate in the form of mixed crystals with barium sulfate cannot be removed by washing.

Place the moist paper with the precipitate in a weighed platinum crucible and place the crucible in a cold electric muffle furnace. Adjust the current so that the furnace attains a temperature of about 800° C. in an hour. With this rate of heating, the paper burns off evenly and completely, without at any time bursting into flame. After an hour, remove the crucible from the furnace, cool in a desiccator, and weigh again. The difference in weight is the weight of barium sulfate plus the coprecipitated barium chromate.

Add to the crucible about 5 grams of anhydrous sodium carbonate and heat strongly in the oxidizing flame of a blast lamp until the precipitate has dissolved in the melt. Remove the flame, and after the melt has solidified, but while it is still hot, place the crucible in a 400-ml. beaker containing about 150 ml. of gently boiling water. When the melt has disintegrated, which takes about 10 minutes, remove the crucible from the beaker and wash thoroughly, pouring the washings back into the beaker. Filter off, wash, and discard the precipitated barium carbonate, collecting the filtrate and washings in a beaker containing about 20 ml. of concentrated hydrochloric acid. The filtrate now contains sulfate, chromate, dichromate, chloride, hydrogen, and sodium ions.

When the solution has cooled, add freshly prepared potassium iodide solution. Titrate with standard sodium thiosulfate solution the iodine set free by the chromate, adding only a slight excess of thiosulfate. Titrate back with standard iodine solution, using starch solution as indicator. Subtract the weight of barium chromate thus obtained from the weight of the mixed precipitate to obtain the weight of barium sulfate.

Discussion of Procedure

In the procedure described, the concentration of hydrochloric acid in the solution to which barium chloride is added is higher than is usually recommended for sulfate precipitations. This was found to be necessary in order to keep the coprecipitation of barium chromate from assuming too large proportions.

A Gooch crucible with asbestos filter cannot be used for the filtration, since when asbestos has been fused in sodium carbonate and the melt extracted with water, the solution contains substances which liberate iodine from potassium iodide. Consequently filter paper (Whatman No. 40) was used and was found satisfactory. Tests showed that chromate was not reduced when ignited in contact with filter paper in a muffle furnace.

Other methods for determining the correction to be applied, in which the precipitate was dissolved in concentrated sulfuric acid prior to further treatment, were tried and found unsatisfactory.

Results

Samples of known weight prepared from the purified reagents were dissolved in water and analyzed by the above

procedure. The quantity of sodium sulfate used was varied from about 0.05 to 0.25 gram, and the ratio of sodium sulfate to sodium chromate was varied from 2.5:1 to 1:75. Table I gives data selected at random from the results obtained.

Columns 1 and 2 give, respectively, the weights of sodium sulfate and of sodium chromate taken. Column 3 gives the weight of the precipitate of mixed barium sulfate and barium chromate obtained. Column 4 gives the weight of sodium sulfate found, calculated on the assumption that all the precipitate is barium sulfate. This quantity, by comparison with the corresponding quantity in column 1, is considerably in error. Column 5 gives the weight of barium chromate in the precipitate, determined as described above. Column 6 gives the weight of sodium sulfate found after the correction has been applied, and column 7 gives the difference between this quantity and the weight of sodium sulfate taken.

It is evident from these results that this method of determining sulfate adequately meets the ordinary analytical requirements.

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Platinized Silica Gel as a Catalyst in Gas Analysis

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Previous work showed that hydrogen, carbon monoxide, and hydrocarbons can be oxidized quantitatively over a platinized silica gel catalyst containing 0.075 per cent of platinum. The commercial catalyst now available contains 0.125 per cent of platinum and is considerably more reactive. The changes in technique from the use of the copper oxide tube give more rapid results, as the catalyst tube replaces the copper oxide tube and slow combustion pipet. Hydrogen is oxidized at 115° C., methane and other hydrocarbons at 510° C. Oxidation temperatures are determined for carbon monoxide, ethylene, acetylene, ethane, and propane. Ethylene may be hydrogenated over this catalyst at 375° C. Nitrous oxide may be determined by reduction with hydrogen at 515° C.

THE previous papers in this series (*2*, *3*, *4*) have shown that hydrogen, carbon monoxide, and hydrocarbons can be quantitatively oxidized by air or oxygen over a platinized silica gel catalyst and have given the conditions necessary for the oxidation. The catalyst used in the previous work, the commercial platinum catalyst of the Silica Gel Corporation, containing 0.075 per cent of platinum, has now been replaced by a more active catalyst containing 0.125 per cent of platinum. This paper states the conditions necessary for its use to replace the copper oxide tube and combustion pipet in the determination of hydrogen, carbon monoxide, hydrocarbons, and nitrous oxide.

Apparatus

The U. S. Steel Corporation gas analysis apparatus used in the previous work was modified by replacing the usual heater for the copper oxide tube with one made from the heating element of a volatile matter furnace (Figure 1). The heating element, *B*, is surrounded by magnesite insulation, *C*, within a sheet-iron shell. The two ends, *D*, are of 0.94-cm. (0.375-inch) Transite, held in place by the straps, *E*.

The technique of the copper oxide tube (*5*) is to cool the tube to room temperature after each analysis. This cannot be done when a catalyst tube is used, as the platinized silica gel will adsorb an appreciable quantity of gases at room temperature. This error is avoided and the time required for an analysis is shortened by maintaining the tube at the temperature of analysis and adjusting the pressure in the tube to the standard pressure after each analysis. It is advisable to keep the catalyst tube

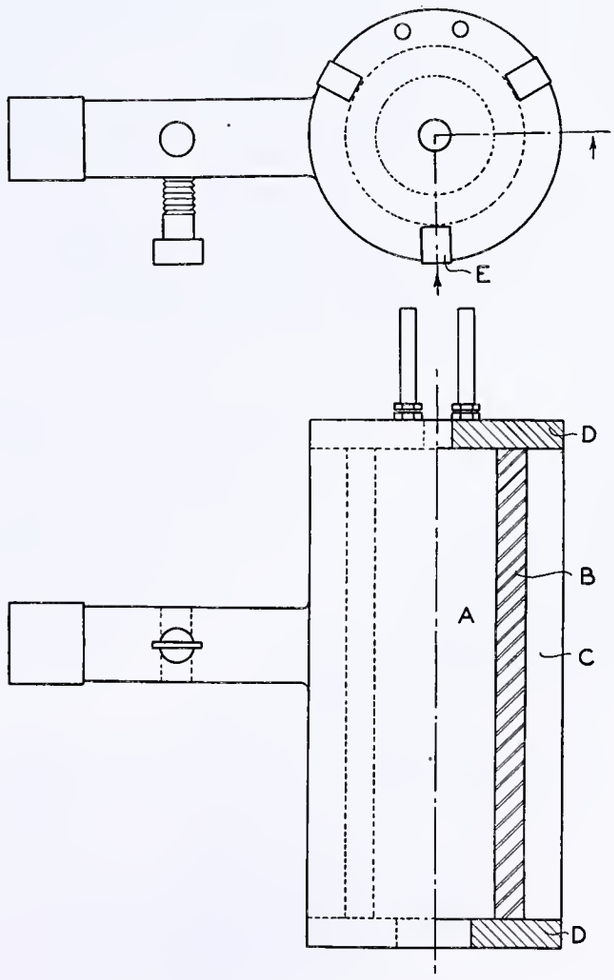

Figure 1. Diagram of Apparatus

Table I. Oxidation of Methane, Ethane, and Propane

Analysis	1	2	3	4	5	6
Oxidation of Methane						
Temperature, ° C.	497	510	510	510	510	510
No. of passes	9	4	6	9	6	6
Rate, ml. per min.	40	40	40	40	40	40
CH_4 by explosion, %	11.2	7.2	7.2	7.2	7.2	7.2
CH_4 by catalyst, %	10.8	6.7	7.3	7.3	7.2	7.2
Oxidation of Ethane						
C_2H_6, %	12.9	..	..	..	..	..
Temperature, ° C.	224	195	170	166	165	145
No. of passes	4	4	4	4	4	4
Rate, ml. per min.	40	40	40	40	40	40
Sample, ml.	92.8	90.4	89.3	88.4	88.3	88.7
Contraction, ml.	1.4	0.9	0.6	0.1	0.0	0.6
CO_2, ml.	1.0	0.2	0.0	0.0	0.0	0.0
Oxidation of Propane						
C_3H_8, %	5.5	..	..	..	..	..
Temperature, ° C.	235	179	150	135	..	..
No. of passes	8	8	6	8	..	..
Rate, ml. per min.	30	30	30	30	..	..
Sample, ml.	98.4	99.2	99.2	95.6	..	..
Contraction, ml.	1.4	1.0	0.0	0.0	..	..
CO_2, ml.	0.2	0.2	0.0	0.0	..	..

open to the buret during heating and cooling, as a large change in temperature (as from room temperature to 510° C.) greatly changes the pressure in the tube.

Methane, Ethane, and Propane

The lowest temperature at which methane could be oxidized completely was first determined. Methane is more resistant to oxidation than the other hydrocarbons, so its oxidation temperature was determined exactly, for here ethane, propane, and higher hydrocarbons will be completely oxidized. The temperature at which oxidation of hydrocarbons begins is of importance, as it shows the temperature below which hydrogen must be oxidized to avoid simultaneous hydrocarbon oxidation. Mixtures of the hydrocarbon, oxygen, and nitrogen were made and analyzed by the explosion method, and this gas mixture was then used in the catalyst tube. Table I shows the results.

The minimum temperature for the oxidation of methane is 510° C. when six double passes are made at the rate of 40 ml. per minute, 100° lower than when a 0.075 per cent platinum catalyst is used. Higher temperatures accelerate the rate of oxidation and fewer passes need to be made. Branham and Shepherd (*1*) have pointed out discrepancies in the explosion method, particularly for ethane. However, the explosion method for methane and nitrous oxide gave concordant results which checked those obtained by the catalyst tube method.

The lowest temperatures at which oxidation of ethane and propane occurred were determined. Table I shows that ethane is not oxidized below 165° nor propane below 150° C. The contraction is greater than should occur with oxidation, or when no carbon dioxide is formed, and is accounted for by adsorption of the hydrocarbon on the catalyst. When the temperature was lowered from 165° to 145° C. an apparent adsorption of 0.6 ml. of ethane occurred, which is much higher than from any ordinary gas mixture because of the 12.9 per cent of ethane in the gas.

Commercial Gas

Hydrogen is oxidized by passing the gas with air over the catalyst at 100° to 110° C. Lower temperatures are not advisable, as the water formed should not be condensed in the catalyst tube.

Carbon monoxide is oxidized by passing the gas over the catalyst at a temperature above 210° C. (Table II); below this the catalyst is poisoned. This temperature is 90° below that found for the 0.075 per cent platinum catalyst. The temperature for the oxidation of carbon monoxide is above the minimum for ethane and propane, so that it cannot be determined by oxidation when these hydrocarbons are present in the gas mixture.

Seattle city gas was analyzed, using three different methods for oxidation. The results (Table III) show that the catalyst tube gives as exact data as the usual combustion methods.

With samples 1 and 2, the hydrogen was removed at 115° C. by five double passes at 40 ml. per minute, and then the hydrocarbons were determined by explosion. With samples 3a and 3b, hydrogen was removed as before, and then the hydrocarbons were determined by six passes through the catalyst tube at 510°. With samples 4a and 4b, hydrogen and hydrocarbons were determined together by six passes at 511° and calculated to hydrogen and methane, which is slightly in error as some ethane is present in Seattle city gas.

Table II. Oxidation of Carbon Monoxide

(Gas mixture 12.7% CO, 18.9% O_2)

Analysis	1	2	3	4	5	6
Temperature, ° C.	320	276	231	210	208	194
No. of passes	6	6	6	6	6	6
Rate, ml. per min.	40	40	40	40	40	40
CO, %	12.6	12.7	12.7	12.6	12.7	7.4

Table III. Analysis of Seattle City Gas

Sample	1	2	3a	3b	4a	4b
CO_2	5.4	5.3	5.3	5.3	5.5	5.5
Ill.	5.7	5.8	5.8	5.8	5.7	5.7
O_2	1.0	1.1	1.0	1.0	1.1	1.1
CO	14.4	14.2	14.2	14.2	14.1	14.1
H_2	41.7	41.8	41.8	41.9	41.9	41.7
CH_4	15.7	15.8	15.8	16.0	16.8	17.0
C_2H_6	0.7	0.6	0.9	0.7	...	...
N_2	15.4	15.4	15.2	15.1	14.9	14.9
Total	100.0	100.0	100.0	100.0	100.0	100.0

Oxidation of Ethylene and Acetylene

Ethylene and acetylene may be oxidized quantitatively with a limited excess of oxygen. Known mixtures of hydrocarbon, oxygen, and nitrogen were made. Ethylene was determined by absorption, acetylene by explosion. The results with the catalyst tube are shown in Table IV.

Ethylene may be oxidized quantitatively above 310°, and acetylene above 325°. Below these temperatures the catalyst is poisoned by the unsaturated hydrocarbon. Whenever the catalyst is poisoned by ethylene, acetylene, or carbon monoxide, it may be reactivated by heating the tube to about 75° above the minimum oxidation temperature and passing air or oxygen through the tube three or four times.

Hydrogenation of Ethylene

In order to determine the possibility of determining unsaturation by hydrogenation, the activity of the platinized silica gel as a hydrogenation catalyst was studied for ethylene (Table V). In oxidizing ethylene, the catalyst was poisoned

Table IV. Oxidation of Ethylene and Acetylene

Analysis	1	2	3	4	5
	Ethylene				
Temperature, ° C.	450	400	326	310	288
No. of passes	4	4	4	4	4
Rate, ml. per min.	40	40	40	40	40
C_2H_4 by absorption, %	11.0	11.0	11.0	11.0	11.0
C_2H_4 by catalyst, %	11.0	11.1	11.0	11.0	5.7
	Acetylene				
Temperature, ° C.	398	338	325	305	305
No. of passes	4	4	4	4	8
Rate, ml. per min.	40	40	40	40	40
C_2H_2 by absorption, %	7.9	7.9	7.9	7.9	7.9
C_2H_2 by catalyst, %	7.8	8.0	7.9	6.3	7.5

Table V. Hydrogenation of Ethylene

(Gas mixture: C_2H_4 16.5, H_2 83.5 per cent)

Analysis	1	2	3	4	5	6
Temperature, ° C.	375	318	318	286	286	286
No. of passes	4	4	8	4	7	10
Rate, ml. per min.	40	40	40	40	40	40
C_2H_4 by hydrogenation, %	16.5	16.0	16.5	15.8	16.3	16.5

Table VI. Reduction of Nitrous Oxide

(Gas mixture: hydrogen, nitrous oxide by explosion 33.7, 33.8%)

Analysis	1	2	3	4	5	6
Temperature, ° C.	518	518	515	491	491	460
No. of passes	6	12	6	4	8	4
Rate, ml. per min.	40	40	40	40	40	40
N_2O, %	33.7	33.7	33.8	33.2	33.6	30.8

below 310°. In hydrogenating ethylene, the reaction proceeds so rapidly at first that the remaining ethylene does not poison the catalyst, so that hydrogenation can be effected as low as 234°, although the reaction becomes extremely slow.

Nitrous Oxide

The reaction $N_2O + H_2 \rightarrow N_2 + H_2O$ can be carried out by explosion technique. Nitrous oxide was mixed with hydrogen and analyzed by explosion. The gas mixture was passed through the catalyst tube and the oxidation temperature determined (Table VI). Nitrous oxide may be determined by making six double passes at 515° or above. Analysis of a cylinder of nitrous oxide for anesthesia showed 99.7 per cent purity.

Summary

A platinized silica gel catalyst containing 0.125 per cent of platinum lowers the oxidation temperature of methane and carbon monoxide by approximately 100° C. from that obtained with a 0.075 per cent catalyst. Temperatures are given for the complete oxidation of carbon monoxide, methane, ethylene, and acetylene and for the start of oxidation for ethane and propane. Nitrous oxide may be reduced over the catalyst by a limited excess of hydrogen at 515° C.

The catalyst tube can replace the copper oxide tube and explosion (or slow-combustion) pipet in any commercial gas analysis apparatus. Large samples may be used without danger of explosion.

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Detection of Certain Metals in Minerals and Ores

An Ammonium Hypophosphite Fusion Method

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When fused with ammonium hypophosphite many minerals and ores are decomposed, the resulting melt often being highly colored. The color of the melt directly, or after being treated with water or hydrogen peroxide, is the basis of the tests described here for chromium, cobalt, columbium, manganese, molybdenum, tellurium, titanium, uranium, vanadium, and tungsten.

Hypophosphites are powerful reducing agents. They decompose upon being heated, giving off hydrogen and phosphine, which ignite. Ammonium hypophosphite decomposes as follows (*1*):

$$7NH_4H_2PO_2 \longrightarrow 2HPO_3 + H_4P_2O_7 + 7NH_3 + 3PH_3 + 2H_2 + H_2O$$

The clear melt that is obtained serves as an excellent medium for showing any color imparted to it by the reduced mineral. The fused mass has a low melting point, about 60° C., and is readily soluble in water. Sodium and potassium hypophosphites are not suitable for this fusion because upon decomposition they form salts with relatively high melting points, instead of the low melting acids that are obtained from the ammonium salt.

Method

About 0.1 gram of the finely powdered mineral is strongly heated in a small evaporating dish with 2 grams of ammonium hypophosphite. The hypophosphite soon begins to decompose and the gases evolved ignite, forming water and oxides of phosphorus. After 2 minutes a quiet fusion mixture is obtained which is used for the individual tests.

Detection of Metals

Cobalt, Titanium, and Tungsten. These three metals give blue melts, but the cobalt melt turns pink upon cooling. For the detection of tungsten, enough water is added dropwise to the warm melt to keep the surface moist. As the water penetrates the melt, the blue color changes to a striking violet. The violet color appears immediately when appreciable amounts of tungsten are present, but 10 to 30 minutes may be required when only very small amounts are present. If the blue color of the melt is due to titanium, the water above the melt becomes a delicate and almost imperceptible rose color. The addition of hydrogen peroxide gives an intense orange-red color. Ammonia causes the rose color to change to blue, but this change is not so sensitive as the color change with hydrogen peroxide. Vanadium also gives a reddish color with hydrogen peroxide, but the vanadium melt is red when hot and green when cool; hence it is easy to distinguish between titanium and vanadium. Since cobalt, titanium, and tungsten are usually not associated together in minerals, these tests are specific for these three metals.

Vanadium, Chromium, and Uranium. These three metals impart a green color to the melt. Vanadium gives a reddish color to the melt when hot, which gradually changes to yellow and finally to green on cooling. The addition of water gives a pale green solution which turns pink when hydrogen peroxide is added. The addition of ammonium carbonate solution to the green melt until the solution is dis-

TABLE I. SENSITIVITY OF TESTS

Material Tested	Weight of Sample	Metal Detected
	Mg.	
Cb_2O_5 + Ta_2O_5 mixture	50	Cb
Columbite (40% Cb_2O_5)	150	Cb
Columbite	100	Cb
WO_3	25	W
Ferberite (6.26% WO_3)	100	W
Ferberite (26.8% WO_3)	30	W
Na_2WO_4	3 (WO_3)	W
Scheelite	120	W
Wolframite	100	W
Hübnerite	100	W
TiO_2	10	Ti
Brookite	100	Ti
Perovskite	120	Ti
Ilmenite	100	Ti
$Co(NO_3)_2$	2	Co
Smaltite	100	Co
V_2O_5	10	V
NH_4VO_3	2	V
Carnotite (1.28% V_2O_5)	200	V
Roscoelite (5.04% V_2O_5)	50	V
Uranyl acetate	4	U
Carnotite (0.28% U_3O_8)	200	U
$Cr(NO_3)_3$	18	Cr
Chromite	100	Cr
MoO_3	4	Mo
Molybdenite (4.07% Mo)	150	Mo
Wulfenite	100	Mo
$MnSO_4$	0.05 (Mn)	Mn
Pyrolusite	50	Mn
TeO_2	10	Te
Sylvanite	100	Te

tinctly basic, followed by the addition of hydrogen peroxide, gives a yellow-orange colored solution if uranium is present. It is better to filter off any solid matter in order to observe the color of the filtrate. Neither vanadium nor chromium gives a yellow-orange color in basic solution. If the green color of the melt is due to chromium, no change in color takes place when hydrogen peroxide is added.

MOLYBDENUM. All molybdenum minerals give a reddish-brown melt, except molybdenite, which is not completely decomposed by the fusion. Therefore, in testing for molybdenum, the melt is treated with concentrated nitric acid, the acid is boiled off, and the mixture is heated to fusion again. The resulting melt is green or blue-green, which changes to yellow upon addition of water.

MANGANESE. Manganese minerals give a clear melt. When concentrated nitric acid is added and the excess acid boiled off, the melt takes on the well-known color of permanganate. Nitric acid does not usually oxidize manganese to permanganate, but in the presence of the melt it does this readily. None of the metals in the usual scheme of analysis interferes with this test for manganese.

TELLURIUM. Tellurium minerals are reduced to metallic tellurium by the fusion and small globules of the metal may be seen floating on the surface of the melt. Strong heating for 2 or 3 minutes causes a deep wine color to appear around each globule of molten tellurium. Addition of water to the warm melt causes the wine color to turn black.

COLUMBIUM. Columbium minerals impart no color to the melt but fine black particles are observed throughout the melt. When concentrated hydrochloric acid is added, the mixture heated to boiling, and a small piece of mossy tin added, an intense blue color develops in a few seconds if columbium is present. Since columbium and tantalum are nearly always associated together in minerals, this test can be used for the detection of both metals.

Sensitivity of Tests

Table I shows that these tests are applicable to different minerals containing the elements for which the tests have been devised, and are sufficiently sensitive to detect appreciable quantities of the metals in question.

It is not claimed that by this method any one of these metals can be detected in the presence of any or all of the others, but it is possible to detect any one metal in any of the naturally occurring minerals thus far tested. Tungsten, for example, can be detected in any one of the tungsten minerals which the authors have been able to obtain. Manganese was detected in a mixture containing 0.5 mg. each of silver, lead, mercury, bismuth, copper, cadmium, arsenic, antimony, tin, cobalt, nickel, chromium, aluminum, zinc, and manganese. During the fusion iron and copper are reduced to colorless compounds which in no way interfere with the tests. If a mineral gives no color, all the metals included in these tests are absent, except columbium and manganese.

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Use of Bromate in Volumetric Analysis

Determination of Arsenic and Antimony Using Internal Indicators at Ordinary Temperatures

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GYÖRY'S method (*1*) for the determination of arsenic and of antimony by titration of strong hydrochloric acid solutions of the trivalent elements, using potassium bromate with methyl orange or indigo sulfonate as internal indicators, requires that the reaction be carried out at 80° to 90° C. The same determination can be carried out at lower hydrochloric acid concentration and at room temperature if the reaction is followed potentiometrically, as shown by Zintl and Wattenberg (*5*). More recently benzopurpurin B was proposed by Raĭkhinshteĭn (*2*) for the determination of antimony using bromate, and Utzel (*4*) suggested a colloidal suspension of alpha-naphtholflavone as a reversible indicator for bromate titrations.

Since the determination of arsenic and antimony using the Györy (*1*) method is still preferred by industrial analytical laboratories and has not been discarded in favor of the Zintl and Wattenberg (*5*) or other procedures, suitable modification of the Györy methods, by means of which the reaction is carried out at ordinary temperatures, has been suggested to the authors. This article describes the use of three internal oxidation indicators of the irreversible type in the Györy procedure, by means of which the oxidation of arsenic and of

Table I. Determination of Bromate Required for Oxidation of 1 Ml. of Internal Indicator in Aqueous Hydrochloric Acid Solution

B. C. I. No.	Indicator Concentration %	Indicator	0.1 N Bromate Required Ml.
88	0.1	Bordeaux	0.08
185	0.2	Brilliant Ponceaux 5R	0.05
246	0.1	Naphthol blue-black	0.03

Table II. Titration of Trivalent Antimony by Bromate at Various Hydrochloric Acid Concentrations

(Bordeaux used as indicator)

Volume of Concd. HCl %	No. of Determinations	Antimony Calcd. Gram	Antimony Found Gram	Error Mg.
35	3	0.1523	0.1523	0.0
25	3	0.1523	0.1522	−0.1
20	3	0.1523	0.1523	0.0
15	3	0.1523	0.1522	−0.1
10	3	0.1523	0.1521	−0.2
5	3	0.1523	0.1523	0.0

antimony by bromate can be carried out at ordinary temperatures and over a wide range, from low to high hydrochloric acid concentrations. The previous papers in the series (*3*) should be consulted for further bromate analytical procedures.

Disadvantage of Irreversible Internal Oxidation Indicators

The organic dyes, Brilliant Ponceaux 5R, B. C. I. No. 185 (0.2 per cent aqueous solution), Bordeaux, B. C. I. No. 88, and naphthol blue-black, B. C. I. No. 246 (0.1 per cent aqueous solution), are irreversible oxidation indicators. Their intense color permits the use of 0.1 to 0.2 ml. of a 0.1 per cent solution for titration, and the potentials at which they are oxidized and the color destroyed, using hydrochloric acid solutions with bromate as oxidant, are above the equivalence point potential for the oxidation of trivalent to pentavalent arsenic or antimony. Their destruction results from the liberation of bromine from the action of the first minute excess of bromate in the presence of hydrochloric acid. The mechanism of the reaction for the destruction of methyl orange or indigosulfonate in the Győry method is the same, except that the reaction mixture must be hot. With all these irreversible oxidation indicators the destruction of the indicator is often premature to a slight extent and the color of the solution fades before the equivalence point of the reaction is reached. While this is troublesome, the required use of additional indicator incurs no appreciable indicator blank in the present case, as shown by Table I. The indicator blank is negligible in all cases. Additional indicator to counteract "fading" can be used without appreciable blank determination.

Reagents Employed

Tenth normal solutions of potassium dichromate, potassium bromate, iodine, and potassium antimonyl tartrate as well as 0.2 *N* sodium thiosulfate solution were made up in accordance with accepted procedures.

The iodine solution was standardized using Bureau of Standards arsenic trioxide (standard of reference No. 83), the sodium thiosulfate was then standardized by liberation of iodine from measured portions of the bromate in 1 *N* hydrochloric acid solution by addition of excess potassium iodide, and the liberated iodine was titrated using the standard thiosulfate with starch as indicator. The antimony solution was standardized using the iodine solution in the presence of excess sodium bicarbonate with starch as indicator in the accepted manner. In all cases calibrated pipets, burets, and flasks were employed and the determinations were made with sufficient duplications to ensure an accuracy of one or two parts per thousand.

Effect of Variation in Hydrochloric Acid Concentration at Ordinary Temperatures

In the titration of the antimony solution with the bromate, the acid concentration was varied over a wide range to study the applicability of the indicator. The volume at the beginning of the titration was 100 ml. in all cases. One or two drops of the indicator solution were used, and by the time the end point was reached most of the indicator had been oxidized by the local excesses of the bromate during its addition. As the indicator fades another drop can be added without causing any error. At the end point the indicator is irreversibly destroyed and the solution becomes colorless. If the fading of the indicator is confused with the end point, another drop of the indicator may be added. If the indicator has faded, the additional drop will color the solution. If the end point has been reached, the additional drop of indicator will be destroyed by the excess bromate in the last drop added.

The results of the titrations of the antimony solution using Bordeaux as the indicator are found in Table II.

The results of the titrations of the antimonyl solution using naphthol blue-black and Brilliant Ponceaux 5R are given in Table III, which also includes the titration of samples of Bureau of Standards arsenic trioxide using Bordeaux.

Bordeaux, Brilliant Ponceaux 5R, and naphthol blue-black are satisfactory indicators for the titration of trivalent antimony at acidities varying from 5 to 35 per cent of concentrated hydrochloric acid, using bromate as oxidant and with titration at ordinary temperatures The same indicators were used in the oxidation of trivalent arsenic, using 20 per cent by volume of concentrated hydrochloric acid, the other conditions being the same. The influence of the fading of these irreversible internal oxidation indicators upon the procedures in question has been discussed and the absence of an appreciable titration error shown. The procedures described are superior to the older method of Győry without requiring titration either in the hot or at higher hydrochloric acid concentrations. At the same time the necessity of a potentiometric titration to gain these advantages as in the Zintl and Wattenberg procedure is avoided.

Table III. Titration of Antimonyl Solution and Arsenic Trioxide

Volume of Concd. HCl %	No. of Determinations	Indicator Used	Antimony Taken Gram	Antimony Found Gram	Arsenic Taken Gram	Arsenic Found Gram	Error Mg.
10, 15, 20, 25, and 35	5	Naphthol blue-black	0.1523	0.1523	...	...	0.0
10, 20, 25, and 35	4	Brilliant Ponceaux 5R	0.1523	0.15225	...	...	−0.05
20	5	Bordeaux	...	...	0.09361	0.09362	+0.05

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Application of a Photoelectric Colorimeter

Determination of Bismuth in Biological Materials

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THE potassium iodide method for the determination of bismuth in biological materials has been used in many forms since first applied by Autenrieth and Meyer (*1*) and modified by Leonard (*4*). It depends upon the formation of a yellow bismuth iodide complex in acid solution and in the presence of a reducing agent. Scholtz and Chaney (*7*), without evaluation of the effect of the reagents on the optical properties of the solution, applied a photoelectric comparator to this method, using standard bismuth solutions for calibration. Baggesgaard-Rasmussen, Jackerott, and Schou (*2*) showed by means of a Koenig-Martens spectrophotometer that the complex involved absorbs blue light up to a wave length of 4500 Å. They found, furthermore, that variation of the acid and potassium iodide within narrow limits had apparently no effect on the light absorption.

In this investigation an attempt has been made to evaluate objectively, by means of a photoelectric colorimeter, the principal factors which affect the application of the iodide method. Revised procedures are proposed for the determination of bismuth in urine, kidney, liver, and muscle, and test analyses are included to indicate the accuracy.

All determinations were made with a photoelectric photometer employing a single cesium cell and standard substitution technique. Approximately monochromatic light was supplied by means of a 6-volt incandescent bulb operating on a storage battery (Exide, Type LXGH), a blue Pyrex glass filter (Corning No. 554 H. R. Lantern Blue), 6.0 mm. in thickness, and a heat filter (Corning No. 396, Light Shade Aklo). The blue filter was chosen because its maximum transmission (*3*) occurs in the region of the prominent absorption band of the bismuth iodide complex (*2*). Other blue filters gave less satisfactory results. Absorption cells of 1.0-cm. or 2.0-cm. depth with optically flat windows were used to hold the solution and the water, respectively. Measurements of the photocurrent were made with a vacuum tube voltmeter working on a 6C6, R. C. A. tube, with a 6E5 tube as null point indicator. This applies the principle of the 6F5 circuit of Müller (*6*). The instrument used in this work gave a precision of ±0.2 millivolt, equal to ±0.1 per cent of the color measured.

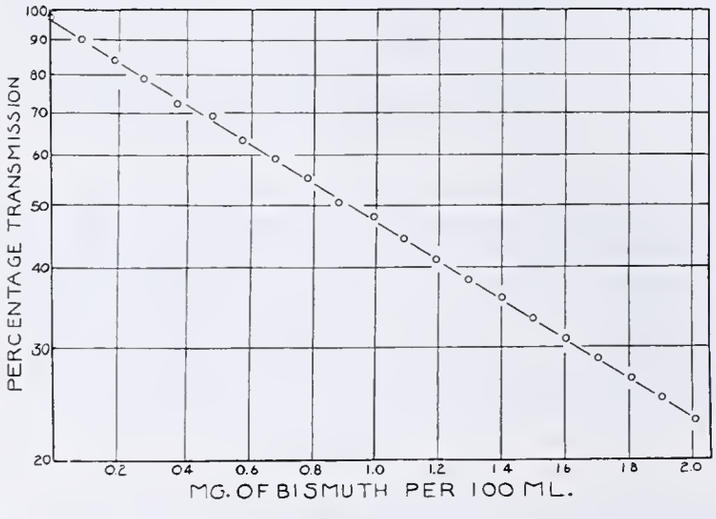

FIGURE 1. RELATIONSHIP BETWEEN LOGARITHM OF PER CENT TRANSMISSION AND BISMUTH CONCENTRATION

For each 100 ml. of solution, 50.0 ml. of 28 per cent (by volume) sulfuric acid, 13.4 ml. of 0.5 per cent Na_2SO_3 solution, and 16.8 ml. of 1.7 per cent KI solution were used with varying amounts of standard bismuth nitrate solution.

Müller (*5*) has shown that the basis for every colorimetric method should be the Lambert-Beer law. In order to establish the justification for the use of this system in a colorimetric procedure the following solutions were prepared, and experiments performed. All reagents were prepared from chemicals of A. C. S. specification.

STANDARD BISMUTH, 0.2321 gram of bismuth nitrate, $Bi(NO_3)_3 \cdot 5H_2O$, dissolved in 50 ml. of 1 to 10 nitric acid and diluted to 1 liter to give a solution containing 10.00 mg. of bismuth per 100 ml.

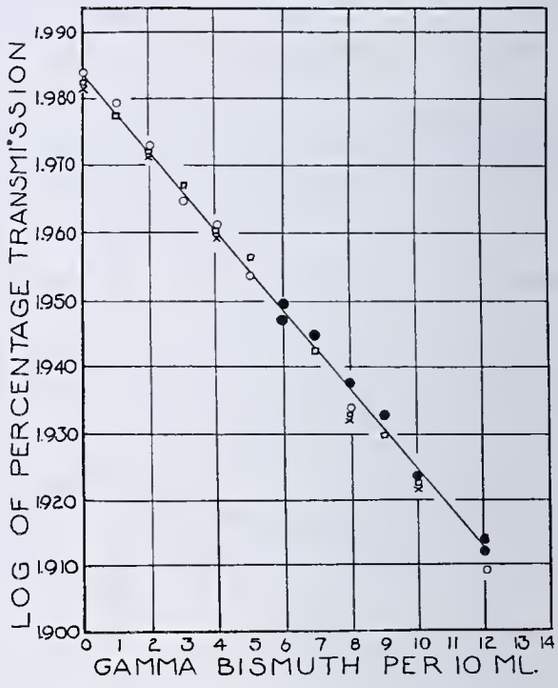

FIGURE 2. RELATIONSHIP BETWEEN LOGARITHM OF PER CENT TRANSMISSION AND 12 MICROGRAMS OR LESS OF BISMUTH

Concentrations of reagents were same as in Figure 1. Cells of 2.0-cm. thickness were substituted for those of 1.0-cm. thickness. Symbols represent determinations made at different times on known concentrations of bismuth. Differences observed at a fixed concentration are a measure of precision of method.

SODIUM SULFITE, 1.00 gram dissolved in water, acidified with 0.8 ml. of concentrated sulfuric acid, and diluted to 200 ml. This solution must be freshly prepared every day.

POTASSIUM IODIDE, 17.0 grams dissolved in 1 liter of water.

SULFURIC ACID, 280 ml. of concentrated sulfuric acid diluted to 1 liter.

Varying amounts of the standard bismuth solution were mixed in each case with 50.0 ml. of sulfuric acid solution, 13.4 ml. of sodium sulfite solution, and 16.8 ml. of potassium iodide solution, and the whole was diluted to 100 ml. in stoppered volumetric flasks. These amounts of reagents were used to get a wide range of bismuth concentration within a measurable transmission. The percentage transmission was then determined for each solution.

Figures 1 and 2 show that the relationship between the logarithm of the percentage transmission and the bismuth concentration is linear; thus, the Lambert-Beer law is obeyed for concentrations up to 2.00 mg. of bismuth per 100 ml. of solution. Figure 2 was prepared as a basis for the determination of from 1 to 10 micrograms of bismuth in 10 ml. of normal blood serum. Absorption cells of 2-cm. thickness and 8-ml.

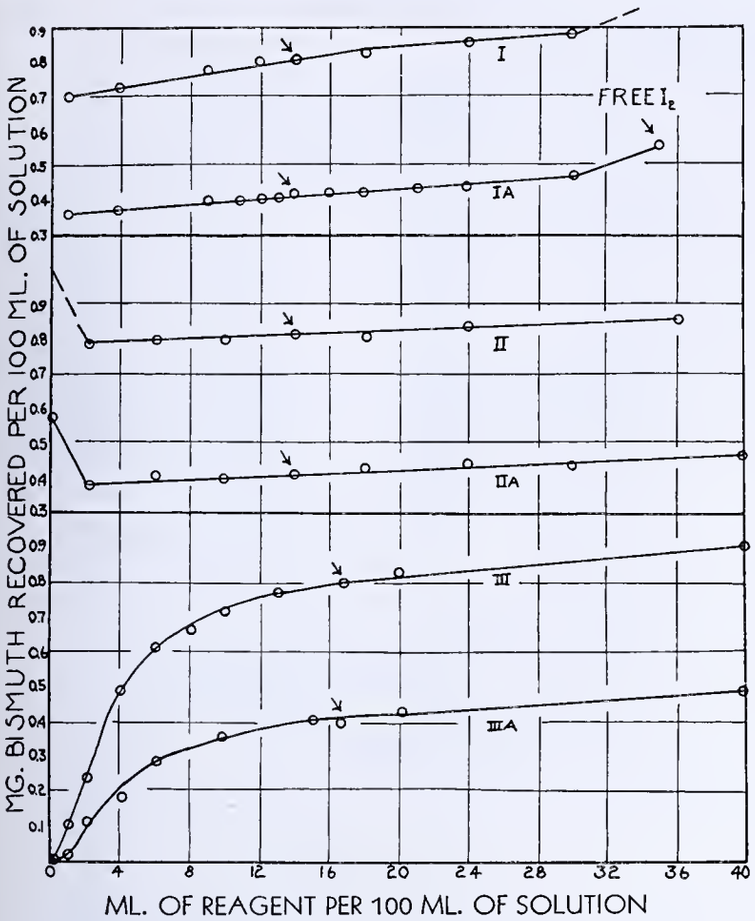

FIGURE 3. INFLUENCE OF VARIATIONS OF REAGENTS UPON RECOVERY OF BISMUTH

One reagent was varied in each curve while others were in same concentrations as in Figure 1.

I, IA. Influence of concentrated sulfuric acid upon recovery of 0.400 and 0.800 mg. of bismuth per 100 ml., respectively

II, IIA. Influence of 0.5 per cent sodium sulfite

III, IIIA. Influence of 1.7 per cent potassium iodide

volume were used. To reduce the accidental errors several measurements were made for each concentration on this curve. The lines do not intercept the axis of ordinates at 100 per cent transmission, because distilled water instead of the reagents (which absorb some light) was used in the reference cell, in order to avoid making up a synthetic mixture of reagents for each determination. Furthermore, if such a mixture were allowed to stand in the open cell it would slowly lose sulfite and free iodine might be liberated. Thus water furnishes a perfectly reproducible reference cell, but by its use no error is introduced into the determination.

To determine the relative effects of the reagents on the color, fixed quantities of standard bismuth solutions were taken, and the concentrations of one of the reagents were varied while all other concentrations were kept the same as in the preparation of the Lambert-Beer's law curve.

Two bismuth concentrations were used for each study—0.400 and 0.800 mg. per 100 ml.—and the results are shown in Figure 3. Curves III and IIIA indicate that the color intensity is a function of the iodide concentration, but as shown by curves I, IA, II, and IIA, it is affected only slightly by the acid and the sulfite. Some sulfite must be present to prevent the precipitation of free iodine, as evidenced by the sudden initial fall (curves II and IIA) in the sulfite curve. If the iodide is in great excess over the sulfite free iodine is liberated, resulting in light absorption. The arrows in Figure 3 indicate the concentrations of the reagents used in obtaining the Lambert-Beer's curve (Figure 1).

To determine the concentration of iron which will interfere, 0.400-mg. and 0.784-mg. samples of bismuth and variable amounts of iron as ferric sulfate were used; the quantities of reagents added were the same as those used in obtaining the transmission curve (Figure 1). Curves IV and IVA, Figure 4, show that the concentration of iron may be as high as 20 mg. without interfering with the determination. Twenty milligrams or less of iron do not cause a change on standing several hours, whereas more than 20 mg. cause the precipitation of iodine.

Separate test analyses with known concentrations of bismuth and various quantities of heavy metals showed that quantitative recovery of bismuth may be obtained in the presence of the following concentration per 100 ml. of heavy metals: 2 mg. of lead, 2 mg. of mercury, 0.5 mg. of copper, and 10 mg. or more of arsenic. As little as 0.5 mg. of silver interferes. Thus it was concluded that the concentration of the above heavy metals normally appearing in tissue will not interfere unless silver is present.

Test analyses showed that, if the solutions are kept securely stoppered after mixing the reagents with the bismuth, the yellow color is stable for as long as 14 days. The same percentage transmission was found at the end of this time as immediately after preparing the color.

Application of the Method to Analyses of Biological Material

URINE. To 100 ml. of urine in a Kjeldahl flask, 10 ml. of concentrated nitric acid and 7.5 ml. of concentrated sulfuric acid are added. This is evaporated to fumes of sulfur trioxide over an open burner. While fuming, a mixture of nitric and perchloric acids (1 to 2) is added dropwise until all color is destroyed. The excess nitric acid is now destroyed by the addition of 1 ml. of 30 per cent hydrogen peroxide, dropwise, to the hot mixture. The peroxide is in turn removed by vigorous boiling. The flask is allowed to cool, about 10 ml. of water are added, and the solution is again evaporated to fumes of sulfur trioxide. The contents of the flask are now rinsed into a 50-ml. volumetric flask, using not more than a total of 25 ml. of water. After cooling, 6.7 ml. of 0.5 per cent sodium sulfite and 8.4 ml. of 1.7 per cent potassium iodide are added and diluted to 50 ml. The solution is centrifuged if not perfectly clear and is then ready for the photometer. The potentiometer is set at zero and the instrument is adjusted to a null point with the phototube dark.

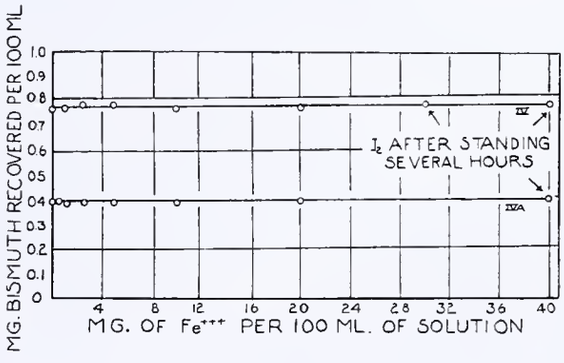

FIGURE 4. INFLUENCE OF IRON, AS FERRIC SULFATE, UPON RECOVERY OF 0.400 AND 0.784 MG. OF BISMUTH PER 100 ML.

The two absorption cells are filled with water, the constant light source is turned on, and the potentiometer is set to a null point. Readings for the right cell, R_0, and the left cell, L_0, are taken alternately by moving them back and forth so as to intercept the beam of light. Three such readings for each cell are, in general, satisfactory. If the readings for the two cells are not the same, a correction is applied in the calculations. The left cell is emptied, rinsed with the solution, and then filled with the solution. Again alternate readings for the unknown, R, and for the water, R_0, are taken.

TABLE I. DETERMINATION OF BISMUTH

Volume of Urine *Ml.*	Bismuth Added *Mg.*	Bismuth Found *Mg.*	Recovery %
Recovery from Urine			
100	0.000	0.000	...
50	0.000	0.000	...
50	0.000	0.000	...
100	0.000	0.003	...
100	0.050	0.051	102.0
50	0.100	0.098	98.0
100	0.100	0.105	105.0
100	0.150	0.148	98.7
50	0.200	0.202	101.0
100	0.250	0.251	100.4
50	0.300	0.301	100.3
100	0.350	0.348	99.4
50	0.400	0.397	99.3
100	0.450	0.445	98.9
50	0.500	0.493	98.6
50	0.600	0.599	99.8
			Av. 100.1
		Mean deviation, %	±1.3
Recovery from Tissues			
Kidney *Grams*			
22	0.000	0.000	...
8	0.000	0.000	...
19	0.100	0.102	102.0
10	0.200	0.204	102.0
9	0.200	0.196	98.0
8	0.300	0.315	105.0
9	0.400	0.393	98.3
21	0.600	0.630	105.0
23	0.800	0.806	100.8
Liver			
20	0.000	0.000	...
23	0.000	0.007	...
25	0.000	0.000	...
27	0.200	0.208	104.0
25	0.300	0.306	102.0
Muscle			
13	0.000	0.002	...
17	0.000	0.001	...
Muscle, Cont'd			
12	0.050	0.048	96.0
13	0.100	0.099	99.0
13	0.150	0.153	102.0
22	0.200	0.200	100.0
13	0.300	0.304	101.3
19	0.400	0.398	99.5
20	0.400	0.405	101.3
15	0.500	0.500	100.0
19	0.600	0.616	102.7
22	0.600	0.617	102.8
17	0.700	0.722	103.1
19	0.700	0.691	98.7
20	0.800	0.788	98.5
24	0.900	0.910	101.1
12	1.000	0.973	97.3
			Av. 100.8
		Mean deviation, %	±1.5
Recovery from Blood Serum			
Blood Serum *Ml.*	*Micrograms*	*Micrograms*	
10	0.0	0.0	...
	0.0	0.3	...
	0.0	0.0	...
	0.0	0.4	...
	0.0	0.0	...
	0.0	0.0	...
	2.0	1.8	90
	2.0	1.9	95
	4.0	4.0	100
	4.0	4.3	108
	6.0	6.2	103
	6.0	5.0	83
	8.0	8.1	101
	8.0	8.6	108
	10.0	9.6	96
	10.0	10.0	100
			Av. 98
		Mean deviation, %	±6

The per cent transmission of the sample is calculated as follows: Cell correction = R_0/L_c, per cent transmission = $R_0/L_0 \times R/R_0 \times 100$, where this R_0/L_0 is usually equal to 1.

The bismuth concentration in mg. per 100 ml. is found by interpolation of the Beer's law curve (Figure 1). The total bismuth content of the sample analyzed is given by:

$$\text{Mg. of Bi} = a \times \frac{b}{100}$$

where a = mg. of bismuth in 100 ml. obtained from the Beer's law curve (Figure 1) and b = total volume to which digested material was diluted.

LIVER, KIDNEY, AND MUSCLE. Not more than 25-gram samples are placed in an Erlenmeyer flask and covered with a volume of nitric acid equal to about twice the weight of the tissue. The flask is heated gently on a steam bath until a clear brown or yellow solution results. On cooling, a layer of fat will usually separate, and is removed by filtering through glass wool into a 500-ml. Kjeldahl flask. The flask and the glass wool are rinsed with nitric acid and the rinsings added to the filtrate. A volume of 7.5 ml. of concentrated sulfuric acid is added to the Kjeldahl flask and the mixture evaporated to fumes of sulfur trioxide. The digestion and estimation are now finished as in the procedure for urine.

BLOOD SERUM. To 10 ml. of normal serum in a 100-ml. Kjeldahl flask, 1.5 ml. of concentrated sulfuric acid and 5 ml. of concentrated nitric acid are added. This is digested as described for urine. By means of 5 ml. of water the residue is rinsed into a 10-ml. volumetric flask and cooled, 1.4 ml. of 0.5 per cent sulfite and 1.7 ml. of 1.7 per cent potassium iodide are added, and the solution is diluted to 10 ml. This is centrifuged and the percentage transmission determined as for urine, substituting cells of 2.0-cm. depth for those of 1.0-cm. depth. The bismuth concentration in micrograms per 10 ml. is found by interpolation of the Beer's law curve (Figure 2).

For the determination of such small quantities of bismuth all glassware used should be first washed with hot nitric acid, all flasks should be kept covered to protect against dust particles, and the rigorous precautions for exact photometry must be followed. Less than 1 microgram of bismuth should not be estimated by the method.

Known amounts of bismuth were added to a series of tissues (cat and rabbit), urine (human), and blood serum (dog) samples. Control experiments using normal tissue, to which

TABLE II. DETERMINATION OF BISMUTH

Total Volume *Ml.*	Bismuth Added *Mg.*	Aliquot Recovery from Urine	Total Bismuth Found *Mg.*	Recovery %
1513	2.50	50	2.54	101.6
		50	2.48	99.2
		50	2.57	102.8
		100	2.51	100.4
		100	2.51	100.4
		100	2.50	100.0
908	2.50	50	2.38	95.2
		50	2.37	94.8
				Av. 99.3
			Mean deviation, %	±2
100	Unknown	10	3.10	...
		10	3.08	...
200	Unknown	10	5.08	...
		10	5.20	...

Total Weight *Grams*	Recovery from Tissue (Liver) *Grams*		
242	25	2.57	...
		2.59	...
262		1.98	...
		1.98	...
291		2.58	...
		2.51	...

no bismuth was added, were also conducted. The analytical results are given in Table I.

The complete procedure was put into the hands of a technician who obtained the results of Table II in duplicate and triplicate on known and unknown samples.

These figures are submitted as evidence that the method is adaptable to clinical use in the hands of a technician. Results on other tissues have been found as precise as these shown.

Summary

By means of a photoelectric colorimeter, the principal factors which affect the application of the potassium iodide method for the determination of bismuth in biological materials were evaluated.

Procedures for a photoelectric determination of bismuth in urine, kidney, liver, muscle, and blood serum have been devised with test analyses included to indicate the accuracy of such procedures.

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Perchloric Acid Oxidation of Organic Phosphorus in Lake Waters

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THE utilization of phosphate, dissolved in lake water, by growing phytoplankton attaches considerable importance to this element and to the phosphorus in the lake. Various studies have shown that only a small portion of the total phosphorus content is present as the readily available phosphate but a large potential supply exists organically bound.

The Denigès (*1*) colorimetric method estimates only the phosphate phosphorus. The amount of organic phosphorus is derived by deducting the phosphate phosphorus from the total phosphorus content. The method as described by Robinson and Kemmerer (*2*) has served very well for the determination of total phosphorus. Briefly, the organic material in a 100-ml. water sample is digested with 0.2 ml. of concentrated sulfuric acid and 0.5 ml. of concentrated nitric acid to convert the organic phosphorus to phosphate. Shortly before sulfuric acid fumes appear 3 ml. of concentrated hydrochloric acid are added to destroy the excess nitric acid.

Although the above method has proved its value, the following alternate procedure is proposed because of its greater simplicity.

To the 100-ml. water sample contained in a 125-ml. Erlenmeyer flask, 0.2 ml. of 72 per cent perchloric acid is added and the sample is evaporated to fumes of perchloric acid. (More acid must be added to hard waters to gain an excess of the acid.) If oxidation of the organic material is slow, as is sometimes the case with water from extractive lakes, the flask may be covered with a watch glass to prevent loss of perchloric acid and the heating continued until the coloration disappears.

The use of perchloric acid as the oxidizing agent has a number of advantages. Only one acid, rather than three, is measured. Undesirable fumes are not inhaled during pipetting or discharged during digesting. The excess nitric acid must be completely removed, as it causes fading of the blue phosphomolybdate complex; the excess perchloric acid does not cause fading. The flask may be covered with a watch glass during digestion to prevent loss of perchloric and phosphoric acids; this cannot be done with the nitric acid because of the retention of the nitric acid. Consequently, with a covered flask there is less need for careful temperature regulation in the digestion to prevent loss of phosphoric acid. The nitric acid is destroyed and expelled at a low temperature, the perchloric acid is present throughout; this means that difficultly oxidizable substances may be subjected to a higher oxidation temperature. Lastly, there is less phosphate impurity in perchloric acid than in the sulfuric-nitric-hydrochloric acid mixture.

The perchloric acid oxidation has proved an efficient method of oxidizing organic phosphorus compounds. First it has been checked by the analysis of Eastman's C. P. glycerophosphate, the phosphorus content of which had been verified by oxidation and gravimetric analysis of macrosamples. Amounts of glycerophosphate approximating the phosphorus content normally found in 100 ml. of lake water were used to simulate lake water conditions. Complete recovery was obtained, as indicated in Table I.

Secondly, the efficiency of the method has been checked by analysis of lake waters which were also analyzed by the sulfuric-nitric-hydrochloric acid method. A comparison of the two methods is included in Table II. Trout Lake water was particularly suited to a critical comparison of the two methods, because in it there was enough extractive organic material to make it more difficult to oxidize than the average lake water. The organic material was as quickly and efficiently oxidized by the perchloric acid as by the nitric-sulfuric acid mixture. During the progress of this

TABLE I. ANALYSIS OF GLYCEROPHOSPHATE

Phosphorus Taken	Phosphorus Found
Mg./l.	*Mg./l.*
0.028	0.029
0.028	0.027
0.028	0.027
0.028	0.027

Table II. Total Phosphorus in Lake Water

	Phosphorus Found	
	$HClO_4$	H_2SO_4-HNO_3-HCl
	Mg./l.	*Mg./l.*
Trout Lake	0.028	0.026
	0.027	0.027
Lake Washington	0.018	0.017
	0.017	0.017
Green Lake	0.024	0.026
	0.026	0.025

Table III. Total Phosphorus in Trout Lake, San Juan Islands, Wash.

	Phosphorus Found	
	Unneutralized	Neutralized
	Mg./l.	*Mg./l.*
$H_2SO_4 + HNO_3 + HCl$	0.024	0.026
	0.024	0.027
	0.024	
0.2 ml. $HClO_4$	0.023	0.028
	0.022	0.027
0.5 ml. $HClO_4$	0.019	0.026
	0.018	

work the oxidation always proceeded quietly and never with violence, probably because of the quantity and nature of the organic compounds present in lake water, together with the gradual oxidation during evaporation and digestion.

It is known that the residual acid from the digestion has a small but definite inhibiting effect upon the formation of the blue reduction complex. This effect may be prevented by neutralization of the excess acid before development of the blue reduction color. Analyses of Trout Lake are given in Table III to show the effect of neutralization. Using either methyl red or phenolphthalein as the indicator, the acid was carefully titrated with *N* ammonium hydroxide, followed by *N* hydrochloric acid until the acid form of the indicator was just prevalent. The colorimetric comparisons were made with the Zeiss-Pulfrich photometer as described by Wirth and Robinson (*3*). Interference by the methyl red was entirely eliminated by the S-72 color filter (average wave length 7200 Å.). Estimations were also made in Nessler tubes when phenolphthalein had been used as the indicator in the neutralization. Although the inhibiting effect of the excess acid is not large, greater accuracy is undoubtedly obtained when the acid is previously neutralized.

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The Mixed Indicator Bromocresol Green–Methyl Red for Carbonates in Water

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The mixed indicator bromocresol green–methyl red has been applied to the detection of the end point of bicarbonate titrations in concentrations which are found normally in natural or commercial waters. Electrometric titration curves for waters of various compositions show that the final equivalence point pH is definitely dependent on the carbon dioxide content there existing. A method is presented whereby the pH value to be obtained at the final end point can be ascertained before that point is reached. This allows the selection of the proper indicator color at the end point. The glass electrode, bromocresol green–methyl red, methyl orange, and methyl orange–xylene cyanole are compared in titrations of carbonates of various concentrations.

THE estimation of carbonates in extremely dilute solution with methyl orange as indicator is unsatisfactory for two reasons: The color change is difficult to observe in solutions whose pH is changing slowly, and the equivalence point in the majority of titrations of this type comes at pH values too high to permit this indicator to respond correctly. The pH at the final equivalence point is a function of the carbonic acid concentration existing there and this in turn is dependent on the neutralized bicarbonate as well as on the initial carbon dioxide content. In the titration of such solutions methyl orange leads to high results if the carbonic acid content at the final equivalence point is lower than about 1.2 millimoles per liter (73 p. p. m. of carbonic acid). This limit is realized if the end point color is read when the indicator shows its first definite cast of orange (pH = about 4.6). For example, if 0.0200 *N* acid is used this indicator shows a minimum positive error of 4.6 per cent for total carbonate and of 7.2 per cent for bicarbonate when used in a solution initially containing 21.6 p. p. m. of carbonate and 17.5 p. p. m. of bicarbonate.

In the official method the carbonic acid end point is determined by the use of methyl orange (*1*, *3*, *4*), methyl orange–xylene cyanole (*3*), or erythrosin (*1*, *5*). Methyl orange is subject to the errors pointed out above and erythrosin requires special technique in its use. For low carbonates, methyl orange–xylene cyanole leads to higher results than does methyl orange alone. Furthermore, the official method using methyl orange has been shown to be unreliable when carbonates are low (*12*). Therefore, an indicator which would respond more nearly at the carbonic acid equivalence point, when solutions of carbonates or bicarbonates are titrated yielding less than 1.2 millimoles of carbonic acid per liter, would prove useful. In such titrations the solutions are fairly well buffered as the equivalence point is crossed. Any indicator which is to reveal this point correctly must respond over a very narrow pH range. A distinct characteristic of mixed indicators is that they tend to show sharp color changes within narrow pH limits.

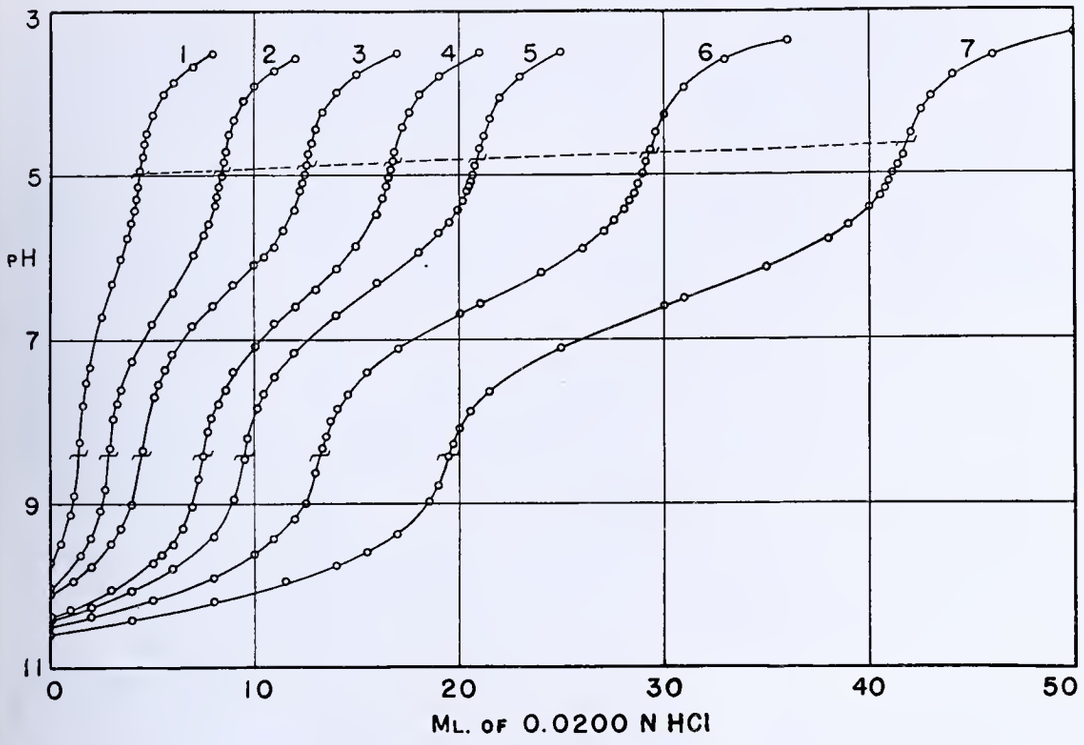

FIGURE 1. TITRATION CURVES OF MIXED CARBONATES

The mixed indicator bromocresol green–dimethyl yellow (*7*) has been recommended for end points in the titration of mixed carbonates. Methyl red and sodium alizarin sulfonate containing certain color screens (*8*) are good indicators for ammonia titrations, showing their maximum response between pH 5.0 and 5.4. Methyl red–bromocresol green (1 to 1) has been recommended as an excellent mixed indicator for titrations in the presence of ammonium ion (*6, 11*). Methyl red (0.05 per cent)–bromocresol green (0.075 per cent) in alcohol (*9, 10*) shows a marked color change near pH 5.1. By lowering the ratio of methyl red to bromocresol green, the maximum response can be made to occur at lower pH values.

Establishment of Equivalence Point pH

A number of solutions containing both sodium carbonate and sodium bicarbonate were prepared and 250 ml. of each were titrated electrometrically with 0.0200 *N* hydrochloric acid (standardized electrometrically against sodium carbonate) using the glass electrode as pH indicator.

The composition of solutions titrated and the equivalence point pH ($pH_{e.\,p.}$) values are recorded in Table I. The entire titration curves are shown in Figure 1.

These data indicate that, whereas the pH at the first equivalence point (bicarbonate) is constant (8.35) over a final range (after titration with 0.020 *N* hydrochloric acid) of bicarbonate from 0.199 to 1.650 millimoles per liter, the pH of the second equivalence point (carbonic acid) changes from 4.98 to 4.63 for final carbonic acid concentrations of 0.197 to 1.525 millimoles per liter, respectively.

The pH values listed in Table I were obtained by plotting $\Delta pH/\Delta ml.$ as a function of ml. of hydrochloric acid, whereby the volume of hydrochloric acid used to the equivalence point was determined, and the pH equivalence point was read from the curves in Figure 1.

TABLE I. ELECTROMETRIC TITRATION OF SODIUM CARBONATE-BICARBONATE SOLUTIONS WITH HYDROCHLORIC ACID

Solution No.	Na_2CO_3	$NaHCO_3$	0.0200 *N* HCl to Equivalence Point HCO_3^-	0.0200 *N* HCl to Equivalence Point H_2CO_3	pH at Equivalence Point HCO_3^-	pH at Equivalence Point H_2CO_3
	Millimoles per liter		*Ml.*	*Ml.*		
1	0.151	0.049	1.89	4.39	8.35	4.98
2	0.231	0.215	2.88	8.45	8.35	4.93
3	0.360	0.286	4.50	12.58	8.34	4.87
4	0.601	0.140	7.51	16.77	8.35	4.83
5	0.765	0.141	9.57	20.90	8.35	4.79
6	1.065	0.212	13.31	29.27	8.35	4.72
7	1.567	0.210	19.59	41.81	8.35	4.63

Selection of Indicator

Since methyl orange or methyl orange–xylene cyanole responds visually at pH values lower than approximately 4.6, the indicator needed must show its response in the range of pH between 4.60 and 5.00. Three drops of a mixed indicator, made by dissolving 0.02 gram of methyl red and 0.10 gram of bromocresol green in 100 ml. of 95 per cent alcohol, when used in either 100 or 250 ml. of solution, show a maximum color change over this pH range. Table II gives the colors which this mixed indicator shows in terms of the pH of the solution; as the pH changes from 5.0 to 4.6 the indicator color changes from light blue or lavender gray to a light pink. With practice, pH values may be determined without comparison to 0.1 pH unit within this range by the use of this indicator.

TABLE II. COLOR OF THREE DROPS OF MIXED INDICATOR IN 100 OR 250 ML. OF SOLUTION

pH of Solution	Color
5.2 and above	Blue with trace of green
5.0	Light blue with lavender gray
4.8	Light pink gray with cast of blue
4.6	Light pink
Below 4.6	Pink or rose

Estimation of pH at Carbonic Acid Equivalence Point

It is possible to estimate the pH at the carbonic acid equivalence point before that point is reached, provided the original solution contains no excess carbon dioxide.

Let the final concentration of carbonic acid equal *C;* then from the dissociation: $H_2CO_3 \rightleftarrows H^+ + HCO_3^-$, it follows that

$$[H^+]_{e.\,p.} = [K_1(C - [H^+])]^{1/2}$$

or

$$pH_{e.\,p.} = \tfrac{1}{2}\,pK_1 - \tfrac{1}{2}\log\,(C - [H^+])$$

If it is assumed $C - [H^+] = C$, then

$$pH_{e.\,p.} = \tfrac{1}{2}\,pK_1 - \tfrac{1}{2}\log C \qquad (1)$$

If the solution at the equivalence point contains 0.196 millimole of carbonic acid per liter (sample 1, Table I) an error of 0.04 pH unit is introduced and if the carbonic acid content rises to 0.431 millimole per liter (sample 2, Table I) the error is only 0.01 pH unit by this assumption. If *B* ml. of mixed carbonate require *D* ml. of *A* normal acid to reach the bicarbonate equivalence point and *X* additional ml. of the same acid to reach the carbonic

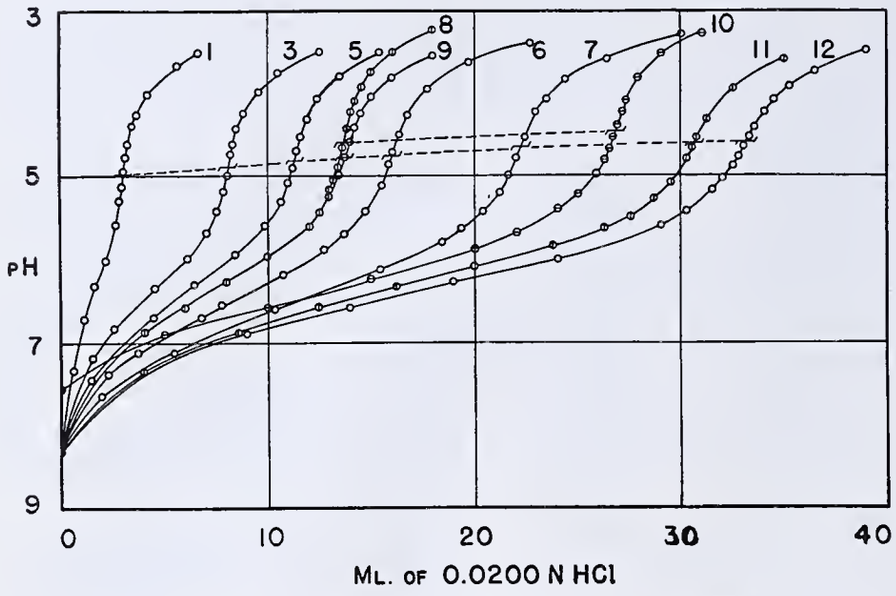

FIGURE 2. TITRATION CURVES FOR BICARBONATES

acid equivalence point, then at the latter $AX = (B + D + X)C$, if all the carbonic acid is developed by titration and no carbon dioxide is lost. Substituting this value of C in Equation 1,

$$pH_{e.\,p.} = {}^1/_2\,pK_1 - {}^1/_2 \log A + {}^1/_2 \log \left(\frac{B+D}{X} + 1\right) \quad (2)$$

If $A = 0.0200\ N$ acid and $pK_1 = 6.50$, Equation 2 becomes

$$pH_{e.\,p.} = 4.10 + {}^1/_2 \log \left(\frac{B+D}{X} + 1\right) \quad (3)$$

Table III shows the results of 24 separate electrometric titrations.

Headings for columns 2, 3, and 4 have the same significance as in Equation 3. The values in column 5 are those in column 4 adjusted to a common basis of 250 ml. of bicarbonate titrated. Columns 6 and 7 show, respectively, the pH at the carbonic acid equivalence point as calculated by Equation 3 and as determined from the titration curves. In Figure 2 titration curves of bicarbonate to carbonic acid for the first twelve solutions of Table III are plotted as milliliters of 0.0200 N hydrochloric acid against pH. In the lower half of Figure 3 the values of experimental $pH_{e.\,p.}$ (column 7 of Table III) are plotted against the values in column 5. The upper part of Figure 3 shows the value of the log term of Equation 3 plotted against the experimental $pH_{e.\,p.}$. The solid curves are those of Equation 3. Values in column 7 of Table III were obtained in the same manner as the values in the last column of Table I.

Samples 1 to 7, inclusive, and 23 represent synthetic mixed carbonates; 10, water from a well at Avon, Mo; 13, 24, and 11, waters from the St. Louis City mains drawn, respectively, on April 18, 1939, November 7, 1939, and January 11, 1940. Samples 8 and 12, 11 plus sodium carbonate and sodium bicarbonate; 9, 100 ml. of 8 diluted to 250 ml. with carbon dioxide-free distilled water; 20 to 22, 11 plus sodium bicarbonate; 14 to 19, 100 ml. of 11 plus sodium bicarbonate diluted to 250 ml. with carbon dioxide-free distilled water. Samples 14 to 18 contained the same amount of sodium bicarbonate.

All samples were run at room temperature (23° to 25° C.) and no precautions were taken to prevent loss of carbon dioxide. Instead, the samples were subjected to the same conditions which prevail during an ordinary titration. Considering these facts, agreement between calculated and measured $pH_{e.\,p.}$ values is very close. This agreement allows Equation 3 to be used to predict the final $pH_{e.\,p.}$ and permits the selection of the proper indicator color for the end point.

TABLE III. COMPARISON OF pH AT CARBONIC ACID EQUIVALENCE POINT

(Calculated with experimental values)

Sample No.	0.0200 N HCl B	D	X	$\frac{250}{B+D}X$	Equivalence Point pH — Equation 3	Experimental ±0.03
	Ml.	*Ml.*	*Ml.*			
1	250	1.39	2.99	2.98	5.07[a]	4.98
2	250	2.88	5.55	5.49	4.93	4.93
3	250	4.50	8.08	7.94	4.86	4.87
4	250	7.51	9.27	9.01	4.83	4.83
5	250	9.57	11.30	10.90	4.79	4.79
6	250	13.31	15.98	15.20	4.72	4.72
7	250	19.59	22.21	20.65	4.66	4.63
12	250	1.00	33.12	32.99	4.57	4.58
8	100	0.40	13.25	32.99	4.57	4.58
9	250	0.30	13.40	13.39	4.75	4.76
10	100	0.00	26.80	67.00	4.44	4.45
11	250	0.00	30.60	30.60	4.58	4.60
13	250	0.00	6.95	6.95	4.88	4.85
14	250	0.00	13.40	13.40	4.75	4.83
15 to 18[b]	250	0.00	13.50	13.50	4.75	4.74
19	250	0.00	14.45	14.45	4.73	4.74
20	100	0.00	12.75	31.97	4.56	4.58
21	100	0.00	13.20	33.00	4.57	4.61
22	100	0.00	13.64	34.10	4.56	4.59
23	100	0.00	21.55	53.87	4.48	4.44
24[c]	250	2.10	10.20	10.12	4.81	4.83

[a] This value should be 5.11, since $C - [H^+] \neq C$ for this solution.
[b] Four separate samples of same composition gave identical results.
[c] Value not included in Figure 3.

TABLE IV. COMPARATIVE TITRATIONS

(Ml. of 0.0200 N hydrochloric acid completely to titrate different waters as determined electrometrically and with mixed indicator, 0.02 gram of methyl red plus 0.10 gram of bromocresol green per 100 ml. of 95 per cent alcohol)

Sample No.		0.0200 N HCl Electrometric	Indicator (arithmetic mean)	Number of Titrations	Deviation of Arithmetic Mean from Electrometric	Maximum Difference from Electrometric	Average Deviation in Single Titration
	Ml.	*Ml.*	*Ml.*		%	%	± *Ml.*
1	250	4.38	4.33	15	1.1	2.3	0.03
2	250	8.43	8.39	12	0.5	1.4	0.03
3	250	12.58	12.53	12	0.4	1.0	0.03
3	100	5.03	5.04	10	0.2	0.4	0.01
4	250	16.78	16.69	11[a]	0.5	0.9	0.02
5	250	20.87	20.85	13	0.1	0.6	0.04
5	100	8.35	8.34	10	0.1	0.5	0.02
7	100	16.72	16.68	5[b]	0.2	0.4	0.01
13	250	6.95	6.85	7	1.4	2.8	0.05
24	250	12.30	12.25	11	0.4	0.7	0.02
25[c]	100	12.52	12.53	7[d]	0.1	0.4	0.03

[a], [b], [d] One value eliminated in each case on the basis that deviation from mean was greater than 4 × average deviation of a single titration. Values eliminated: [a], 16.86; [b], 16.58; [d], 12.42.
[c] Sample not included in Table III. Synthetic carbonate-bicarbonate.

Titrations with Mixed Indicator

Samples of bicarbonates and of mixed carbonates as well as waters from the St. Louis City mains were titrated using 2 drops of 1 per cent phenolphthalein followed by 3 drops of mixed indicator. If the solution assumed a pink color with phenolphthalein, titration was carried on until the last trace of pink was discharged. Three drops of mixed indicator were added to the solution reacting neutral to phenolphthalein and the titration was continued to the end point. The color of the mixed indicator at the end point was taken as that consistent with the $pH_{e.\,p.}$ read from the curve in the lower part of Figure 3. All titrations were made with 0.0200 N hydrochloric acid (standardized electrometrically against sodium carbonate) with the solutions in beakers resting on a white background. The indicator color change is best observed by looking downward through the solution. If the solution is placed in a white porcelain casserole or evaporating dish the end point is more distinct. Results of these titrations as compared to the electrometric values are shown in Table IV.

In order to compare the results obtained by use of the mixed indicator with those given with methyl orange and methyl

orange–xylene cyanole, six synthetic sodium carbonate solutions were titrated using each of the three indicators (Table V). End points for methyl orange (*3*) were taken when the first definite shade of orange was observed and those for methyl orange–xylene cyanole (*3*) when the first trace of gray appeared in green. When phenolphthalein was used in each sample it became colorless at a volume of acid approximately one half of that used when methyl red–bromocresol green began to change in color. One half of the total methyl red–bromocresol green titer, when referred to the curve in the lower half of Figure 3, gave the following approximate $pH_{e.\ p.}$ values for the separate solutions: 4.85 for 30 and 50 p. p. m. of carbonate; 4.75 for 70 and 100 p. p. m.; 4.6 for 150 p. p. m.; and 4.5 for 250 p. p. m. Therefore with methyl red–bromocresol green the following end point colors were taken: in 30 and 50 p. p. m. of carbonate the lavender cast was discharged and a pink developed on a background of light blue; in 70 and 100 p. p. m. the bluish cast was discharged leaving a pinkish gray; in 150 p. p. m. the pinkish gray was converted to a pink; and in 250 p. p. m. the pinkish gray was converted to pink and the solution vigorously stirred for 30 seconds, during which time a bluish cast appeared. The removal of carbon dioxide during stirring causes the pH to rise to about 4.8, which is within the range of the indicator. This color was then converted to pink. (If this solution was again stirred for 30 seconds a grayish pink developed which could be converted to pink with 1 or 2 drops of acid. Therefore, one vigorous stirring of 30 seconds removed sufficient carbon dioxide to allow the indicator, methyl red–bromocresol green, to give the correct end point in the titration of the 250 p. p. m. of carbonate.)

The hydrochloric acid used in these titrations was standardized by diluting 0.8835 gram of pure sodium carbonate to 1 liter with carbon dioxide–free distilled water, diluting 10.00 and 25.00-ml. portions of this solution to 100 and 250 ml., respectively, with carbon dioxide–free distilled water, and titrating both electrometrically and in the presence of the mixed indicator. Results: electrometric, 8.35 and 20.87 ml.; using mixed indicator, 8.34 and 20.85 ml. of hydrochloric acid. Normality of hydrochloric acid; electrometric, 0.01996; using mixed indicator, 0.0200.

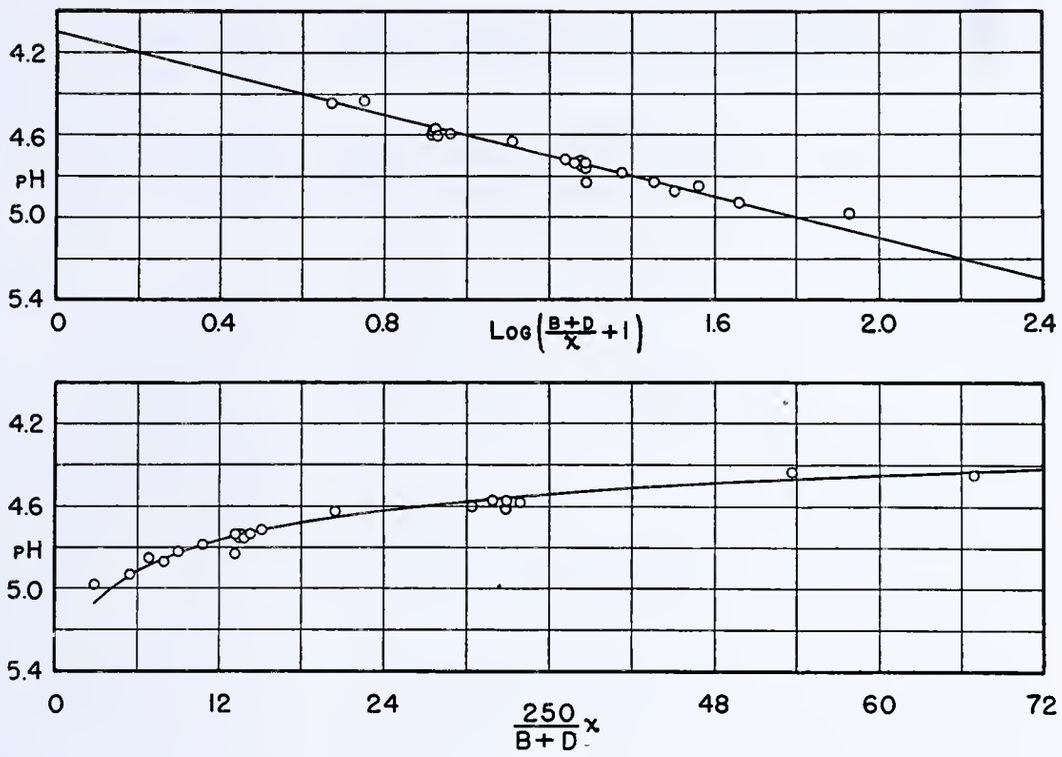

FIGURE 3. EQUIVALENCE POINT pH IN MIXED CARBONATE TITRATIONS

Discussion of Results

Figures 1 and 2 and especially Figure 3 show that, if the carbonic acid equivalence point is to be determined with reasonable accuracy in very dilute solutions, titrations must be carried to pH values consistent with the carbonic acid concentration.

Each electrometric titration was carried out with 3 drops of mixed indicator in the solution and for each sample whose $pH_{e.\ p.}$ was higher than approximately 4.60 the indicator showed a color change as the equivalence point was crossed. In samples 10 and 23 ($pH_{e.\ p.}$ = 4.45 and 4.44, respectively) the indicator developed a pink before the equivalence point was reached. Sample 10, which contained excess carbon dioxide initially, gave a $pH_{e.\ p.}$ value higher than would have been expected if all carbon dioxide had been retained by the solution. In samples 11, 20, 21, and 22 the indicator passed from light pink gray to light pink as the equivalence point was approached.

The differential method applied to the titration curves gave $pH_{e.\ p.}$ values which could be estimated to 0.03 pH unit. Variations in these values from those calculated by Equation 3 are caused by at least two factors: loss of carbon dioxide during titration, and error in estimation of the value.

The method used for estimating the $pH_{e.\ p.}$ before it was reached may be best shown by quoting a specific example.

During one of the titrations of 250 ml. of sample 4 of Table IV, the color of 2 drops of 1 per cent phenolphthalein was discharged with 7.50 ml. of 0.0200 *N* acid and 3 drops of the mixed indicator had lost its green and developed a cast of lavender (pH = 5.0 from Table II) with 16.30 ml. of acid. From Figures 1 and 2 it can be seen that at this pH the volume of acid added is within 0.5 ml. of the equivalence point. The values B = 250, D = 7.50, and X = 8.8 (approximately), when substituted in Equation 3, give $pH_{e.\ p.}$ = 4.84. From Table II it is seen that the color to be expected at a pH of 4.8 is a light pink gray with a

TABLE V. TITRATION WITH DIFFERENT INDICATORS

(Comparison of methyl red–bromocresol green, methyl orange, and methyl orange–xylene cyanole indicators in titrations of carbonates with 0.0200 *N* HCl)

CO_3^{--} in water, p. p. m.	30	50	70	100	150	250
Water titrated, ml.	250	100	100	100	50	50
HCO_3^- developed, p. p. m.	29.8	48.6	67.2	93.9	136	210
0.0200 *N* HCl required, ml.	12.50	8.33	11.67	16.67	12.50	20.83
Methyl red–bromocresol green, ml. of HCl, mean[a]	12.55(19)	8.33(15)	11.67	16.70(10)	12.52	20.86
Error, %	+0.4	0	0	+0.2	+0.2	+0.2
Maximum deviation from mean, ml.	0.08	0.03	0.02	0.08	0.04	0.04
Methyl orange[b], ml. of HCl, mean[a]	13.06	8.48	11.73	16.74(6)	12.51	20.85
Error, %	+4.5	+1.8	+0.5	+0.4	+0.1	+0.1
Maximum deviation from mean, ml.	0.18	0.07	0.09	0.06	0.05	0.03
Methyl orange–xylene cyanole[c], ml. of HCl, mean[a]	13.17	8.60	11.92	16.92	12.68	20.96
Error, %	+5.3	+3.2	+2.1	+1.5	+1.4	+0.6
Maximum deviation from mean, ml.	0.17	0.03	0.08	0.11	0.03	0.01

[a] Arithmetic mean of five titrations unless indicated by number of titrations in parentheses.
[b] End point taken when first definite shade of orange appeared.
[c] End point taken when first trace of gray appeared in green.

cast of blue. Titration was continued to this quality of color and the buret reading was 16.72 ml. The arithmetic mean of 11 such titrations was 16.69 ml. The electrometric value for a similar sample was 16.78 ml. at $pH_{e.\ p.} = 4.83$.

The need for the selection of the proper $pH_{e.\ p.}$ is shown clearly by a comparison of curves 8 and 9 of Figure 2. Both represent the titration of 100 ml. of a certain water: curve 8, for the water without initial dilution, and curve 9 for the water diluted initially to 250 ml. with carbon dioxide–free distilled water. The measured $pH_{e.\ p.}$ for 8 is 4.58 and for 9 is 4.76. An end point at pH of 4.76 for 8 would come 0.15 ml. too early and at a pH of 4.58 for 9 would come 0.15 ml. too late, each of which corresponds to 1.1 per cent error. Samples 8, 10, 12, and 23, all of which show $pH_{e.\ p.}$ values lower than 4.60, should be diluted with carbon dioxide–free distilled water before titration or the indicator should be converted to pink, the solution vigorously stirred for about 30 seconds, and then the blue cast, which develops during agitation, converted to pink. The samples may be titrated, without dilution or agitation, using methyl orange as indicator.

Table IV shows the accuracy with which titrations with the mixed indicator agree with the corresponding electrometric values. It will be noted from column 6 that, of the eleven samples titrated, only 1 and 13 show a deviation of the arithmetic mean greater than 1 per cent from the electrometric value. The values in column 7 represent the maximum difference (a single determination) shown by any titration from the respective electrometric value. Of the 113 titrations represented in Table IV, no value obtained differed from the electrometric value by more than 2.8 per cent.

The titration data recorded in Table V show that the mixed indicator is more reliable than either methyl orange or methyl orange–xylene cyanole except in solutions in which the bicarbonate developed is high. The bicarbonate is the component which is titrated to the final end point. It is apparent from Table V that the error of the mean for titrations in the presence of mixed indicator is less than 0.5 per cent for all concentrations of bicarbonate titrated. The corresponding errors for methyl orange as indicator are greater than 0.5 per cent for solutions developing bicarbonate less than about 67 p. p. m. The errors are greater for all solutions in which methyl orange–xylene cyanole was used.

The hydrochloric acid used was standardized electrometrically and with mixed indicator. The indicator method gave a normality which was within 0.2 per cent of the electrometric value. The concentration of sodium carbonate used for standardization corresponded to 50 p. p. m. of initial carbonate. If the acid had been standardized with methyl orange its normality would have been 0.01966 (from values in Table V), which would have given an error in the mean of +2.7 per cent for 29.8 p. p. m. of bicarbonate and of −1.6 per cent for 210 p. p. m. of bicarbonate. If the acid is standardized against sodium carbonate, with methyl orange, where the carbonate is 70 p. p. m. or greater it may be used for solutions in which the bicarbonate at the phenolphthalein end point is 67 p. p. m. or greater, but not for more dilute solutions without the introduction of a positive error. Therefore one should standardize the acid against a solution containing approximately the same concentration of bicarbonate at the phenolphthalein end point as the solution which is to be titrated when methyl orange is used. Methyl orange–xylene cyanole is less desirable from this standpoint than methyl orange alone.

The curve in the lower half of Figure 3 explains the reason this type of variation is to be expected. The final $pH_{e.\ p.}$ is a function of the bicarbonate titrated and no indicator can show the correct end point unless the indicator color is selected to correspond to the correct $pH_{e.\ p.}$ for the solution in question, or unless the final pH value is adjusted to that to which the indicator responds. The final pH may be raised considerably by removal of carbon dioxide from solution. Thus, the solution developing bicarbonate of 210 p. p. m. has a final $pH_{e.\ p.}$ of about 4.5, which is outside the range of the mixed indicator. Vigorous agitation of the solution for 30 seconds removed sufficient carbon dioxide to allow the pH to rise into the range of the indicator. The mixed indicator has not been applied to more concentrated solutions but should function if carbon dioxide is removed. This could be accomplished by agitation of the solution until 1 or 2 drops of acid are required to discharge the cast of blue which develops on stirring.

Samples which contain free carbon dioxide (react colorless to phenolphthalein and alkaline to methyl orange, or the mixed indicator) are titrated to the phenolphthalein end point with sodium carbonate (*4*) or sodium hydroxide (*2*) solution to determine the carbon dioxide content. The resulting solution in either case can be titrated with 0.020 *N* hydrochloric acid from the phenolphthalein to a mixed indicator end point to determine the total bicarbonate present in the solution at the phenolphthalein end point. All this bicarbonate may have been developed by reaction of sodium hydroxide or sodium carbonate on carbon dioxide, although some may have been present in the original solution. The milliequivalents of hydrochloric acid minus the milliequivalents of sodium hydroxide or sodium carbonate will give the milliequivalents of bicarbonate in the original solution.

The mixed indicator can be used to detect the end point in all titrations of bicarbonate with acid provided the $pH_{e.\ p.}$ is not lower than 4.60. If materials are present which show buffer action other than that of bicarbonate with carbonic acid the above principles will not apply. The indicator, however, does show the correct end point for phosphates (pH = 4.6) and may be used in their presence.

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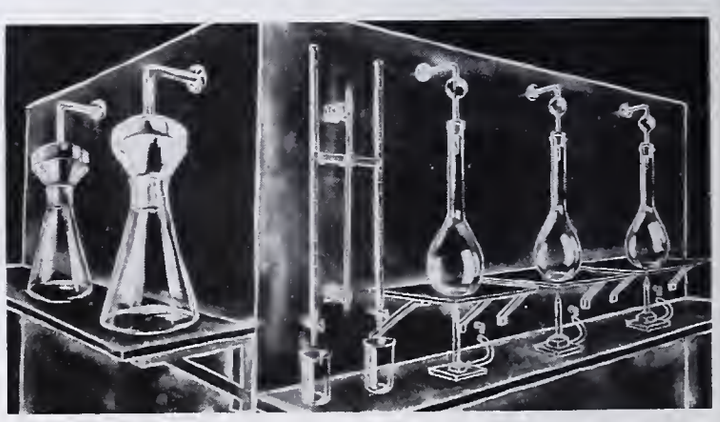

Studies on Soybean Carbohydrates

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Neither the identity of all carbohydrates occurring in soybeans nor their percentage distribution has yet been well established. Evidence that this is so is to be found in the scanty data in the literature, in the diversity in manner of reporting carbohydrate forms, and in the lack of agreement of figures given. This paper presents analytical data on amounts of sugars, galactans, and pentosans found in several edible varieties of soybeans at different times during their maturation from a very immature green vegetable stage to the mature seed. The three carbohydrates mentioned were determined because preliminary data indicated that they might be interrelated during growth. Total carbohydrates and total and reducing sugars tended to decrease as the beans became more mature, while galactans and pentosans tended to increase.

Qualitative separation of hemicellulose fractions was attempted by methods which have heretofore been employed with woody and herbaceous materials. The relatively large amount of protein present in soybeans interfered greatly in this procedure, but evidence was obtained that the hemicelluloses of soybeans are a mixture of galactoarabans containing galacturonic acid.

A SUMMARY is given in Table I of such data on the percentages of different carbohydrates in soybeans as could be found in the literature. Since each investigator has specified certain carbohydrate forms, some of the data are scarcely comparable. In addition, other workers have identified certain of the carbohydrates—for example, Kraybill, Smith, and Walter (*12*) isolated crystalline sucrose from soybeans. Tanret (*21*, *22*) claimed to have separated and identified stachyose. Herissy and Sibassié (*11*) indicated the presence of stachyose and raffinose. Although the authors have never identified starch in soybeans by microscopical methods, it has been reported by others to be present in amounts varying from none to 5 per cent or more, as determined by chemical analysis. Certainly the percentage of starch is highly insignificant in comparison with the quantities present in Lima, navy, and other kinds of beans. In the present investigation, sugars, galactans, and pentosans were determined, since preliminary data (*26*) indicated that these carbohydrates might be interrelated during growth.

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Analyses for Sugars, Pentosans, and Galactans

MATERIAL. The soybeans used were grown by the Agronomy Department of the University of Illinois, at Urbana. Sugar analyses were run on beans of the 1936 and 1937 crops; analyses for pentosans and galactans on those of the 1938 crop. The seven varieties for which sugars, pentosans, and galactans are being reported included Emperor, Funk Delicious, Hokkaido, Imperial, Jogun, and Willomi, six varieties rated "very good" for edible purposes by Woodruff and Klaas (*25*), and also Illini, a field variety which received a rating of "good" from the same investigators. Sugars only are being reported for Fuji and Higan, which also received a rating of "good".

Samples were analyzed at four stages of maturity. The very immature stage was designated as "precooking". The "cooking" stage refers to beans which could be shelled out and used as green vegetables, similar to peas. The "postcooking" stage is that in which the beans were too old to be suitable as green vegetables, but were not yet mature. The fourth was the mature stage.

PREPARATION OF SAMPLES. The green samples were blanched in the pods 2 minutes to facilitate shelling, as well as to arrest enzyme activity. They were cooled immediately under running water, shelled out at once, and weighed. Two 100-gram samples of shelled beans of each variety were preserved for sugar analyses by boiling for 10 minutes in 500 ml. of 75 per cent alcohol, to which 3 grams of calcium carbonate had been added to keep the pH sufficiently high to prevent hydrolysis of the complex sugars. The samples were cooled, stoppered, and stored in the dark until analyses were made.

The green shelled soybeans to be used for pentosan and galactan analyses were immediately ground through a Russwin No. 1 handmill, and dried at once for 48 hours at 80° C. They were then reground to pass 32-mesh, weighed, extracted for 24 hours by refluxing with petroleum ether (b. p. 30–60° C.) to remove oil, dried, weighed, and reground to pass 100-mesh.

The mature beans were prepared for sugar analysis by merely grinding to pass a No. 32 grit gauze. Otherwise the mature beans were treated like the green beans—i. e., ground to pass 32-mesh, oil-extracted, and reground to pass 100-mesh.

All samples used for pentosan and galactan determinations were treated as follows: Portions of approximately 5 grams each were weighed accurately into 250-ml. centrifuge bottles. Each was extracted four times with distilled water, to remove proteins; four times, 1 hour each, with boiling 80 per cent alcohol, to remove sugars; four times, 0.5 hour each, with cold 10 per cent alcohol to remove dextrins; and four times, 0.5 hour each, with boiling water, to remove any residual dextrins, etc. The wash liquor was centrifuged off after each extraction. The last three sets of extractions were those recommended by the Com-

TABLE I. PERCENTAGES OF CARBOHYDRATE CONSTITUENTS OF SOYBEANS

Form of Carbohydrate Reported	Summary of Analytical Data Given: Street and Bailey (*20*)[a]	Burrell and Wolfe (*5*)[b]	Bailey, Capen, and LeClerc (*4*)[c]	Woodruff and Klaas (*25*)[d]	Satow (*18*)[e]	Chiu (*6*)[f]	Gerhardt (*10*)[g]
Sugars	..	...	7.0	7.18–9.62	...	..	11.12
Sucrose	3.31	4.0–6.39	..	..	5.90	..	..
Invert sugar	0.07	...	..	..	..	..	..
Reducing sugars	..	0.053–0.229	..	Trace	..	..	..
Dextrose after hydrolysis	..	3.27 –4.45	..	..	..	..	..
Stachyose	..	...	..	..	3.52	..	..
Raffinose	1.13	0.86–1.12	..	..	..	..	..
Starch	0.50	0–0.16	5.6	0.0	..	..	..
Dextrins	3.14	2.39–5.53	..	..	..	..	2.84
Pentosans	4.94	2.51–3.24	4.4	..	3.80	4.41–5.24	..
Galactans	4.86	1.15–2.08	..	..	4.62	..	..
Total hemicellulose	..	...	..	..	..	..	5.95
Undetermined hemicellulose	0.04	...	..	..	..	..	..

[a] Hollybrook variety, 12.6 per cent moisture.
[b] Five edible varieties.
[c] Average of many varieties, 8 per cent moisture.
[d] Fourteen edible varieties, 7 per cent moisture.
[e] Analyses by Yakawa.
[f] Four varieties.
[g] Varieties not stated, 5.36 per cent moisture.

TABLE II. PERCENTAGES OF SUGARS, PENTOSANS, AND GALACTANS IN SOYBEANS DURING MATURING

(Vacuum-dried basis)

Variety of Soybeans	Stage of Maturity	Total Carbohydrates (by Diff.) 1936	Total Carbohydrates (by Diff.) 1937	Total Sugars 1936	Total Sugars 1937	Reducing Sugars, 1937	Pentosans, 1938	Galactans, 1938
Emperor	Precooking	...	...	...	...	..	3.05	1.21
	Cooking	...	41.67	...	18.27	5.66	2.39	2.02
	Postcooking	...	...	...	...	..	3.65	2.13
	Mature	...	43.91	...	9.11	..	3.53	2.03
Funk Delicious	Precooking	45.63	45.88	25.45	26.54	8.11	2.71	1.55
			44.25		20.30	6.84		
	Cooking	42.89	41.93	21.34	16.04	5.22	2.43	2.68
		38.87	39.66	17.93	16.80	5.13		
		38.01		14.83				
	Postcooking	35.17	39.17	13.90	16.24	5.03	2.91	2.35
			38.72		13.28	3.98		
	Mature	32.41	33.35	10.35	10.28	..	3.66	2.56
Hokkaido	Precooking	42.94	46.37	26.90	19.44	7.42	2.38	1.38
	Cooking	42.75	44.31	20.67	19.44	6.97	3.09	1.84
		39.07	44.49	16.44	18.29	6.39		
	Postcooking	35.71	41.69	9.02	14.19	5.63	3.78	3.14
			36.36		14.55	5.34		
			34.77		14.07	4.89		
	Mature	33.31	37.53	9.40	7.56	..	3.79	2.60
Imperial	Precooking	...	...	...	...	...	2.36	1.30
	Cooking	40.48	38.98	16.28	17.19	5.28	2.98	1.71
	Postcooking	...	...	...	...	..	2.81	2.55
	Mature	32.23	35.94	10.32	9.46	..	3.65	2.26
Jogun	Precooking	...	...	...	...	...	2.46	1.37
	Cooking	31.68	49.70	12.38	16.94	5.98	2.64	1.79
	Postcooking	...	...	...	...	..	3.39	2.24
	Mature	33.86	37.26	9.96	9.02	..	3.73	1.99
Willomi	Precooking	44.87	43.84	31.66	27.26	9.70	2.50	1.20
	Cooking	42.91	42.25	22.01	22.83	7.40	2.98	2.24
		42.23	37.24	20.02	20.21	6.31		
		35.56	33.00	14.76	15.38	4.96		
	Postcooking	33.28	32.19	10.24	14.76	4.45	3.58	2.33
		34.02	31.94		14.27	4.23		
	Mature	31.70	35.69	9.27	9.79	..	3.40	2.72
Fuji	Precooking	38.44	51.27	23.34	20.70	6.60	..	..
		36.58		21.19				
	Cooking	37.27	49.64	17.40	18.88	6.05	..	..
		34.75	46.54	16.42	16.68	5.15		
	Postcooking	31.61	35.76	15.29	14.39	4.52	..	..
			36.50		5.94	1.77		
	Mature	35.47	36.82	8.78	8.01	..	..	..
Higan	Cooking	34.33	43.92	11.66	17.85	5.66	..	..
	Mature	34.94	35.18	10.29	8.49	..	..	..
Illini	Precooking	47.01	47.72	21.22	16.46	5.78	2.58	1.34
	Cooking	39.41	46.61	19.20	16.37	5.39	2.43	1.81
		39.20	38.60	15.25	14.64	4.79		
	Postcooking	35.22	42.18	12.30	13.08	3.98	3.76	1.51
			39.55		9.23	1.80		
	Mature	31.08	40.50	10.34	9.11	..	3.58	2.21

mittee on Methods of Chemical Analysis of the American Society of Plant Physiologists (*23*, *24*). These exhaustive extractions were sufficient to have removed all sugars. From the nature of the extraction liquors used, it is felt that the probability of the loss of the two polysaccharides at this stage was slight. Loss would have caused low final values, furthermore, and the actual values obtained are well within the range of those reported by other investigators.

DETERMINATION OF SUGARS. The alcohol was drained from the green soybeans, as the first extract. The beans were ground in a mortar and extracted with boiling 70 per cent alcohol for 1 hour. The suspension was cooled and filtered. This was repeated three times. The five extracts were combined, and evaporated on a water bath to remove the alcohol. The alcohol-free extract was clarified with neutral lead acetate and diluted to volume by the official method (*3*, pp. 134–5). The mature beans were extracted four times and the combined extracts treated as above. Sugars were determined according to the Lane and Eynon titration method (*3*, p. 477). Reducing sugars were determined on the clarified solutions. Aliquots of the solutions were inverted with hydrochloric acid (*3*, p. 341) before determination of total sugars. All sugar determinations were run in duplicate. The amount of reducing sugars present in mature soybeans was so small that no results could be obtained with this method.

DETERMINATION OF PENTOSANS AND GALACTANS. Methods of determination were those chosen by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists (*23*, *24*). Galactans were determined by Dore's method (*8*), which differs from the official method in omitting solution and reprecipitation of the mucic acid. The precipitate obtained was found to be pure mucic acid, as indicated by analysis and by melting point. Pentosans were determined by the official method (*3*, pp. 344–5), by which they are calculated from the weight of furfural phloroglucide obtained after distillation of the furfural. It was necessary, however, because of excessive foaming, especially of immature samples, to substitute a 500-ml. distilling flask for the 300-ml. flask designated by the official method. All analyses were run in quadruplicate.

DETERMINATION OF TOTAL CARBOHYDRATES. Total carbohydrates were calculated by difference after moisture, protein, fat, and total ash of soybeans had been determined.

DISCUSSION OF ANALYTICAL DATA. Analytical data are shown in Table II. The cooking stage is that at which the soybeans are suitable for use as green vegetables, similar to peas. Where two or more values are reported for one type of determination at a given stage, the data are in chronological order for samples of increasing maturity.

While there was some variation in constituents of soybeans grown in different years, the fluctuations were no greater than would be expected with samples not grown under identical conditions. Comparison of the 1936 and 1937 values for total sugars and for total carbohydrates shows that the fluctuations, in general, were not greater from year to year than from variety to variety. Unpublished preliminary data show this to be true, also, for pentosans and galactans.

Both reducing and total sugars decreased with increasing maturity. The changes were often marked even during the comparatively brief period during which the beans remained in the cooking stage. The total sugar content of the soybeans when in the green vegetable stage was but little less than that of shelled green peas, and was nearly twice that of the mature soybeans.

Attention is called to the average percentage of total sugars found in soybeans at different stages of maturity. Even on a moist, fresh basis the amount of sugar in green soybeans is about 4.2 per cent of the total weight. There is reason to believe that most if not all of these sugars can be assimilated by the body. In fact, Adolph and Kao (*1*) reported that about 40 per cent of the total carbohydrates of soybeans could be utilized by the animal body. According to this statement, 14 per cent by weight of mature dry soybeans (40 per cent of the 35 per cent of total carbohydrates) represents assimilable carbohydrate. This fact is significant in view of a popular tendency to speak of soybeans as a "diabetic food". The term implies that soybeans are essentially carbohydrate-free, and has been used especially to designate the low starch content of soybeans. The term "diabetic food" has itself lost its earlier significance since the advent of insulin therapy.

In general, both pentosan and galactan content increased with increasing maturity of the beans. It seems probable that the decrease in sugars with increasing maturity is directly related to the simultaneous increase in pentosan and galactan content. The high values for pentosans obtained at the precooking stage of Emperor, Funk Delicious, and Illini varieties are as yet unexplained. The fact that other varieties studied (unpublished data) showed this peculiarity makes it improbable that the analytical data are affected by some undetermined experimental factor. Pringsheim (*17*) states that the pentosan content of seeds decreases with age. It is possible, therefore, that a portion of the pentosans and galactans may be metabolized during the maturing processes. Several varieties showed a decrease in one or both of these constituents from the postcooking to the mature stage. The general increase in pentosans and galactans with increasing maturity of the soybeans would, however, be expected if these compounds were functioning as reserve carbohydrates.

Plants of the same variety grown under different conditions often show distinct variations in chemical constituents. Plants of the same species, but of different varieties, exhibit even greater variations. Both types of variations are illustrated by the data obtained in this investigation. The sea-

Table III. Summarized Data on Percentages of Soybean Carbohydrates Found

(Vacuum-dried basis)

Stage	Total Carbohydrates (by Diff.) Range	Av.	Total Sugars Range	Av.	Reducing Sugars Range	Av.	Pentosans Range	Av.	Galactans Range	Av.
Precooking	38.44–51.27	44.57	16.46–31.66	23.37	5.78–9.70	7.41	2.36–3.05	2.58	1.20–1.55	1.34
Cooking	31.68–49.70	40.60	11.66–22.83	17.41	4.79–7.40	5.76	2.39–3.09	2.71	1.71–2.68	2.01
Postcooking	31.61–42.18	36.11	5.94–16.24	12.80	1.77–5.63	4.15	2.81–3.78	3.41	1.51–3.14	2.32
Mature	31.08–43.91	35.36	7.56–10.35	9.38	..[a]	..	3.40–3.79	3.62	1.99–2.72	2.34

[a] Too low for analysis by method used.

sonal differences were as great as the varietal differences. At maturity, the one stage which could be determined without subjective observations, and, therefore, the one at which the beans were most strictly comparable, there was a 24 per cent variation from the average for total carbohydrates, 10 per cent for total sugars, 5 per cent for pentosans, and 16 per cent for galactans, for the samples studied.

The general trends are strikingly shown by the ranges and averages in Table III. Although in every case there is an overlapping of the limits from stage to stage for any given determination, when the averages are compared there is a regular decrease for total carbohydrates, total sugars, and reducing sugars, and a similar, though less marked, increase for pentosans and galactans through the successive stages from precooking to mature.

Comparison of Tables I and III will show that the average value found for pentosans in mature soybeans is lower than values reported by Street and Bailey (*20*), by Bailey, Capen, and LeClerc (*4*), and by Chiu (*6*). The value is only slightly lower than that given by Satow (*18*), and appreciably higher than those found by Burrell and Wolfe (*5*). Both Street and Bailey (*20*) and Satow (*18*) reported galactans in nearly twice as great amount as was found by the present writers, whose results, however, are considerably higher than the values reported by Burrell and Wolfe (*5*).

Qualitative Separation of Hemicellulose Fractions of Soybeans

Chemical analyses for pentosans and galactans give only partial information about the hemicellulose complex of soybeans, since many hemicelluloses are known to be polyuronides, similar to pectin. Pectic substances may themselves occur in as important amount as hemicelluloses. It was hoped that a qualitative separation of the hemicellulose fractions might throw further light on the nature of that portion of soybeans to which is assigned the name "carbohydrates".

Other workers have shown that pectic compounds are related to softening changes which vegetable tissues undergo as they are cooked. Personius and Sharp (*16*) state "that the intercellular material of potato tissue is pectic in nature and that the softening of tissue characteristically obtained during cooking is the result of an extensive decrease in cell adhesion which accompanies the weakening of the intercellular cementing substance". Simpson (*19*) found that the tissues of carrots and parsnips tend to disintegrate during cooking, and that this disintegration follows the change of protopectin to pectin. The relation of hemicelluloses to such a disintegration seems possible in view of the fact that hemicelluloses often have structural as well as reserve functions in the plant (*13*).

Soybeans soften noticeably less than many kinds of plant tissue when they are cooked to a degree that makes them acceptable for eating. This point was thought to lend added interest to a study of their hemicellulose fractions.

Extraction. Mature Willomi soybeans of the 1939 crop were used. The beans were ground through a Hobart mill to pass 32 mesh, and were then fat-extracted by refluxing with petroleum ether, boiling point 30° to 60° C., for 24 hours. After evaporation of the ether, the beans were reground through the Hobart mill to pass 100 mesh.

The pectin and hemicellulose extractions were based upon the methods of Norris and Preece (*15*), with certain modifications suggested by Norman (*14*). The method as finally adopted for this work was as follows:

The 100-mesh sample was extracted twice, for 2-hour periods, on the steam bath with 0.5 per cent ammonium oxalate. The extraction liquors were centrifuged off and combined, the calculated amount of acetic acid was added to precipitate protein, which was centrifuged off, and the pectin was then precipitated by the addition of alcohol, washed, and dried in the usual manner. The residue was suspended in 2 per cent sodium carbonate solution and extracted on the steam bath for 4 hours. The extraction liquor, containing a portion of the hemicelluloses, was centrifuged off and reserved. The residue was suspended for 15 minutes in 1500 ml. of distilled water with 75 ml. of "Purex" (5.25 per cent sodium hypochlorite) and 30 ml. of 20 per cent sulfuric acid added, then centrifuged. The residue was extracted on the steam bath for 35 minutes with 0.5 per cent sodium hydroxide, filtered hot through glass wool, and the residue was extracted at room temperature for 18 hours with 0.5 per cent sodium hydroxide. After filtration through glass wool, the 0.5 per cent sodium hydroxide extracts were combined with the 2 per cent sodium carbonate extract.

A series of tubes was set up with varying ratios of acetic acid to combined hemicellulose extracts, to determine the optimum amount of acid to be used for precipitation of hemicellulose A, a procedure recommended by Angell and Norris (*2*). Hemicellulose A was precipitated by addition of the calculated amount of glacial acetic acid. The crude hemicellulose A was washed four times with distilled water, and dried in the usual way with alcohol and ether. It was worked out of ether on a porous plate and dried over phosphorus pentoxide in a vacuum desiccator.

After the A fraction had been removed from the combined hemicellulose extracts, a half volume of acetone was added to the solution to precipitate hemicellulose B. There was practically no precipitation at this point. After clarification with the aid of Filter Cel, another volume of acetone was added, equal to that used to precipitate hemicellulose B. This precipitated hemicellulose C, which was washed and dried like the A fraction.

The solution from which hemicellulose fractions A, B, and C had been removed was treated with a large excess of acetone. A precipitate was obtained which was designated "fraction D".

From 500 grams of soybeans (7.82 per cent moisture), 14.38 grams of crude hemicellulose A were obtained, 0.16 gram of crude hemicellulose C, and 1.24 grams of the D fraction. The amount of pectin, precipitated from the 0.5 per cent ammonium oxalate extracts, was so slight as to be practically negligible, but it is possible that pectin was lost during removal of the protein. Other series of extractions gave similar results.

The fractions accounted for only about one half of the hemicelluloses present, according to the analytical data previously given. The methods of extraction, precipitation, and fractionation, however, have never been considered to be strictly quantitative. For material with as many interfering components as are encountered in soybeans, further refinement of the methods will be necessary before more nearly quantitative recovery of hemicelluloses can be anticipated. No hemicelluloses, as these are defined by Norman (*13*, p. 37), would have been removed prior to the alkaline extractions. The losses were doubtless due to incomplete extraction.

In spite of the preliminary extractions, considerable protein was carried over into the hemicellulose extracts, as much as 30 per cent in some cases, and was precipitated with the various fractions.

Purification. The crude hemicellulose A was dissolved (5 grams to 500 ml.) in 4 per cent sodium hydroxide. Glycerol and cupric sulfate were added to precipitate the A_1 fraction, according to the method of Angell and Norris (*2*). No precipitate of

A_1 was obtained. Upon neutralization with acetic acid, a voluminous precipitate of A_2 separated. Repeated washings with acetone failed to break down the copper complex. Alcohol washings proved equally unsuccessful. After solution in 4 per cent sodium hydroxide and reprecipitation, repeated several times, the copper-hemicellulose complex was still persistent. It could be decomposed with acetic acid, but on reprecipitation the copper was still in combination with the hemicellulose. No method has yet been found which will completely free the hemicellulose from copper. This is probably due to the protein content of the hemicellulose fraction. A portion of the crude hemicellulose A, therefore, was treated with pepsin. After the treatment and subsequent washing and drying, 4.38 grams of crude hemicellulose A yielded 3.19 grams of "purified" hemicellulose A (72.83 per cent), which was still highly impure, containing 13.5 per cent of nonprotein nitrogen.

Three grams of crude hemicellulose C, similarly treated with pepsin, yielded 2.56 grams of the "purified" product (85.33 per cent). The sample was not large enough to furnish material for nitrogen determination, but undoubtedly considerable quantities of protein degradation products were present in it also. Preliminary experiments, using glycerol and cupric sulfate to effect precipitation, showed no C_1 fraction present. The precipitate of hemicellulose C_2 was a copper-hemicellulose complex as difficult to break up as the A_2 complex described above. The C_2 fraction was, therefore, treated with pepsin, washed, and dried, as the A_2 fraction had been.

Identification. Each hemicellulose fraction was hydrolyzed by heating with hydrochloric acid. The solutions were then tested by heating with concentrated sulfuric acid, by heating with phloroglucinol, by treatment with potassium permanganate, and by heating with Disch's reagent, to determine which sugars had been produced by the hydrolysis of each fraction (*7*). Osazones were also prepared.

A portion of each solution was heated with basic lead acetate to test for the presence of galacturonic acid (*9*). This test was not satisfactory; it was very difficult to check results upon the samples. Portions of the solution were heated with 12 per cent hydrochloric acid and the evolved gases tested for presence of carbon dioxide, to determine the presence of uronic acids (*13*, p. 64).

Results and Conclusions. Hemicelluloses A_2, C_2, and the D fraction each contained galactose, arabinose, and uronic acid. The uronic acid was undoubtedly galacturonic acid, as this is the acid usually occurring in the presence of galactose. Both galactose and uronic acid contents progressively increased in proportion to arabinose from A_2 to C_2 to D fraction, as indicated by the speed of reaction, etc., of the qualitative tests used. Although the test used for galacturonic acid was not satisfactory, it gave positive results with the A_2 fraction.

It seems probable that the soybean hemicelluloses comprise a mixture of galacto-arabans containing galacturonic acid. This is a combination frequently encountered (*13*, p. 37). The three fractions obtained are considered to be portions of this mixture which have been separated arbitrarily on the basis of their physical properties.

Only a very small amount of pectin was obtained by precipitation from the ammonium oxalate extract, but it is possible that pectin was lost in the precipitation of the protein. A low content of pectic compounds, which other workers (*16*, *19*) have shown to be concerned in the softening of vegetable tissues during cooking, may explain the firmness which persists in soybeans even after they are cooked.

Summary

The sugar, pentosan, and galactan contents of edible varieties of soybeans were determined at different stages of maturity.

Both reducing and total sugars decreased with increasing maturity. Pentosan and galactan contents tended to increase as the soybeans matured. It is suggested that the decrease in sugars may be related to the increase in pentosans and galactans, and that the latter function as reserve carbohydrates in the seeds.

In the soybean varieties studied, both seasonal and varietal differences in carbohydrate constituents were marked.

The hemicelluloses of Willomi variety soybeans were extracted and fractionated. The large percentage of protein present in soybeans rendered purification exceedingly difficult. Fractions A_2 and C_2 were obtained, as well as a D fraction precipitated by excess acetone from the liquor from which A_2 and C_2 fractions had been removed. Qualitative tests indicated that each of the three fractions obtained was composed of arabinose, galactose, and a uronic acid, undoubtedly galacturonic acid. The ratio of galactose and uronic acid to arabinose appeared to increase from A_2 to C_2 to D fraction.

The hemicelluloses of the soybean are considered to be a mixture of galacto-arabans containing galacturonic acid. The fractions obtained differed in physical properties and in relative proportions of the constituents.

A low pectin content of soybeans may explain their failure to soften greatly during cooking.

Acknowledgments

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Micro-Kjeldahl determinations on the hemicellulose fractions and analysis and melting point of the mucic acid obtained during galactan determinations were run by courtesy of the United States Regional Soybean Industrial Products Laboratory, Urbana, Ill.

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A Pendulum Method for Measuring Settling Velocities

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IN THE preparation and study of liquids containing finely divided particles it is frequently desirable to know how fast the particles are settling out of the suspension. This is especially true when a stable mixture or a mixture which is expected to endure for a long time is being investigated. It is a relatively simple matter to measure the settling rate when the liquid involved is transparent, for then one needs only to observe the rate at which the surface between the clear liquid and the suspension is moving. If, however, the liquid is opaque, the task of measuring the settling rate is a difficult one and a new method of measurement needs to be devised.

To meet this problem of opaque suspensions, a method was developed in this laboratory in which the stability of a suspension and the rate of particle fall could be obtained from a few relatively simple measurements. The method utilized the principles of a compound pendulum and was an extension of a method first introduced by Manning and Taylor (*1*), which was somewhat limited in its scope, since it was employed only to detect settling and did not measure the rate. In the present method it was possible to measure not only the rate at which the center of gravity of the mixture moved, but also the speed with which the individual particles fell. The liquid with its suspended particles was placed in a long tube and mounted so that the tube swung like a pendulum from a support just above the center of gravity of the suspension. When the particles settled, the center of gravity of the system was lowered, causing the period of oscillation to shorten. The tube was also equipped with a second support just above the first one and the periods of oscillation were measured for both supports. The data on these periods for various times of settling were then used to calculate the rate at which the center of gravity of the entire mixture was lowered, which gave a measure of the suspension's stability.

It was also possible by making certain simplifying assumptions regarding the nature of the settling process to calculate the rate at which the particles themselves were settling. In developing the theory for this calculation, it was assumed that all the particles in the suspension fell with the same constant velocity, that there was no interference, and that the particles did not agglomerate. While it is generally known that these effects occur to some degree in most cases of settling, they were ignored in this instance to keep the mathematical theory as simple as possible. Since the experimental results for the samples investigated agree reasonably well with the derived equations, there is some justification for the simple picture of the settling process as adopted in this analysis.

The double support pendulum method as described here was developed primarily to study suspensions of finely divided coal particles in fuel oil, but has also been used to measure the settling of milk in cream and the settling of paint pigments.

To facilitate the calculations of settling rates when a great number of samples are being studied, certain approximate expressions for these velocities have also been developed.

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Experimental Procedure

The essential parts of the apparatus consisted of a 100-ml. Nessler tube fastened in a holder which in turn was suspended from one of its supports by means of two sharp points resting on a flat plate (Figure 1). The holder, which was 32 cm. long, was made of brass and either one of two sets of points could be used as the support. The pendulum unit was enclosed in a constant-temperature box equipped with an observation window. This box also served to eliminate errors due to air currents. The period of oscillation was measured with a stop watch.

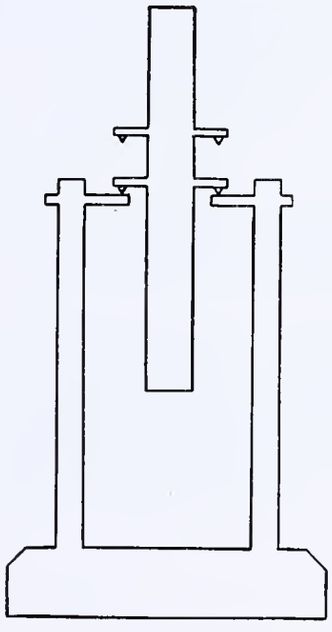

FIGURE 1. DIAGRAM OF PENDULUM SHOWING BASE AND TWO SETS OF SUPPORT POINTS

The oils used in this investigation were virgin gas oil, cracked gas oil, and several cracked residue fuel oils. The coal was washed Kansas bituminous, ground so that all passed a 200-mesh screen. Mixtures of 10 to 50 per cent coal by weight were investigated.

The oil-coal mixtures were poured into the Nessler tube until the surface level was about the same distance above the lower axis of suspension as the axis was above the bottom of the tube. As the coal particles settled, the periods of vibration were measured for both support axes. These measurements were made at 0.5-hour intervals, which were later lengthened to 1-hour and 2-hour intervals. Readings were stopped when the measured periods showed no further change, since it was apparent the particles had completely settled.

Theory of Stability Determinations

The period of vibration, T, for a compound pendulum is given by

$$T^2 = \frac{4\pi^2 I}{MgH}$$

where I is the moment of inertia of the pendulum about its support axis, M its mass, g the acceleration due to gravity, and H the distance from the axis of suspension to the center of gravity. Since a well-known theorem of mechanics states that the moment of inertia about any axis, I, is related to the moment of inertia about the center of gravity, I_0, by the expression

$$I = I_0 + MH^2$$

where H is the distance between the gravity and support axes, the period about the lower axis T_2 may be expressed as

$$T_2^2 = \frac{4\pi^2 (I_0 + MH^2)}{MgH}$$

and about the upper axis, T_1, as

$$T_1^2 = \frac{4\pi^2 I_0 + M(H + h)^2}{Mg(H + h)}$$

where h is the distance between the two support axes. Subtracting these two expressions and solving for H gives

$$H = \frac{4\pi^2h^2 - ghT_1^2}{-8\pi^2h - g(T_2^2 - T_1^2)} \quad (1)$$

The period for the lower axis, T_2, is greater than that for T_1 and, as the settling proceeds, decreases much more rapidly than T_1. H increases with settling and at a rate which is proportional to the lowering of the center of gravity of the suspension.

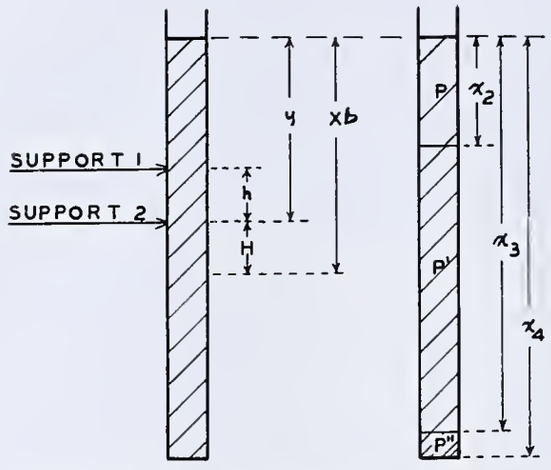

FIGURE 2. SCHEMATIC DIAGRAM OF PENDULUM SHOWING IMPORTANT DISTANCES AND DENSITIES OF LIQUID DURING SETTLING

To find how x'', the center of gravity of the mixture, and H are related, let x'' be the distance from the surface of the liquid to the center of gravity of the liquid mixture, xa the distance from the surface to the center of gravity of the holder, and xb the distance between the surface and the center of gravity of the holder and liquid mixture together. Also let M' be the mass of holder and liquid mixture, M'' the mass of the holder, m' the mass of the suspended particles, and m the mass of the liquid. Then, taking moments about the surface as an axis,

$$M'xb = M''xa + (m' + m)x'' = M'(H + y) \quad (2)$$

where y is the distance from the bottom axis to the top of the liquids (Figure 2). Combining Equations 1 and 2 yields

$$(m' + m)x'' = \frac{(4\pi^2h^2 - ghT_1^2)M'}{-8\pi^2h - g(T_2^2 - T_1^2)} + M'y - M''xa \quad (3)$$

This gives at once the center of gravity of the mixture in terms of the measured periods of vibration. The center of gravity of the holder may be determined in a separate experiment with an empty holder in the same manner that x'' is determined above for the holder filled with the liquid mixture.

Sample Data on Stability

An example of the results obtained is shown in the reproduction of the data for a 10 per cent mixture of coal and virgin gas oil.

The measured period, T_2, is plotted in Figure 3 and a smooth curve drawn through the experimental points. At 8.5 hours T_2 reached a constant value and remained so for readings taken up to a total elapsed time of 57.5 hours. T_1 happened to be constant for this sample and had the value of 1.40 seconds.

In this experiment the total mass of holder and mixture M' was 181.33 grams, of the holder M'' 114.52 grams, and of the mixture $(m' + m)$ 66.81 grams. Period measurements with the empty holder gave a value of 12.96 cm. for xa. The distance between supports h was 2.06 cm., and the distance between the surface of the liquid mixture and the lower support, y, was 12.90 cm.

From the smooth curve of Figure 3, new values of T_2 were taken, and by substitutions in Equation 2, x'' was calculated. The calculated x'' values, representing the distances from the liquid surface to the center of gravity of the mixture, are graphically shown in Figure 4.

In this sample with its low concentration of coal, the center of gravity of the system was not altered much, even when settling had been completed. This sample was obviously not stable, having completely settled out in 8.5 hours.

Theory of Velocity Determination

In setting up the expression for the velocity of settling, it was assumed that the suspended particles fell with a uniform velocity. At some time, t hours after settling begins, the particle-free liquid extends to x_2 and the layer of settled matter has risen to x_3, both distances being measured from the surface of the liquid (Figure 2). If p'' stands for the linear density of the settled layer, p' the linear density of the mixture, and p the linear density of the particle-free oil, moments about the liquid surface may be taken, giving

$$(m' + m)x'' = {}^1/_2px_2^2 + {}^1/_2p'(x_3 - x_2)(x_3 + x_2) + {}^1/_2p''(x_4 - x_3)(x_4 + x_3)$$

If v stands for the velocity of fall, then $x_2 = vt$ and $x_3 = x_4 - kvt$. Here k is the ratio between the rates at which the two surfaces, x_2 and x_3, move and it can be shown that $k = p'c/p''$, where c is the concentration of coal in the mixture.

If x_2 and x_3 are replaced in the above equation by these expressions involving the velocity and time, the equation becomes

$$(m' + m)x'' = e + fvt - qv^2t^2 \quad (4)$$

where
$$e = {}^1/_2p'x_4^2$$
$$f = (p'' - p')kx_4$$
$$q = {}^1/_2(p' - p) + {}^1/_2(p'' - p')k^2$$

Equation 4, when used with Equation 3, permits the evaluation of k and v for each measured T_1 and T_2. In measurements on 10 per cent virgin oil, $p'' = 4.4$ grams per cm., $p' = 2.63$ grams per cm., $p = 2.54$ grams per cm., and $x_4 = 25.4$ cm. The values of f and q which best fit Equations 3 and 4 gave for k in this investigation a value of 0.0442. The calculated data on the velocity of settling for various time intervals are shown in Figure 5. The velocities are constant and give no evidence of a trend with time, showing that the assumption regarding uniform velocity of fall which was employed in the derivation was justified. Two points on the curve fall outside the rest and were discarded in determining the average velocity, which was 2.36 cm. per hour.

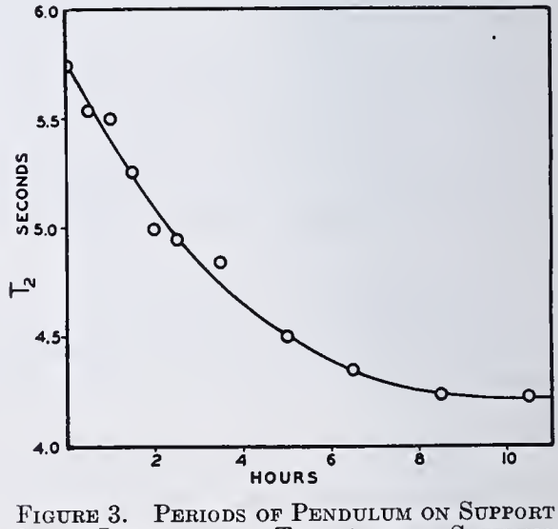

FIGURE 3. PERIODS OF PENDULUM ON SUPPORT 2 FOR INTERVALS OF TIME t AFTER SETTLING BEGINS

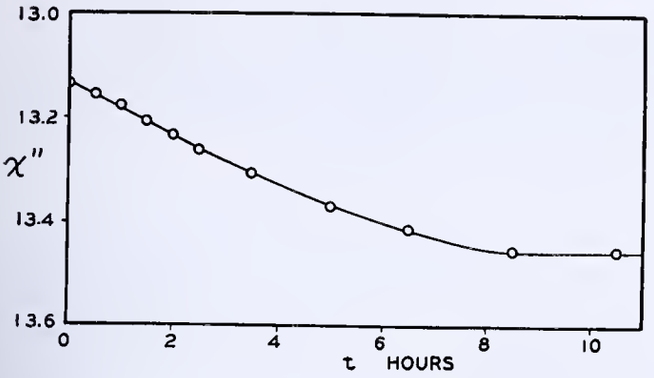

FIGURE 4. LOWERING OF CENTER OF GRAVITY OF LIQUID WITH TIME OF SETTLING

Sensitivity of Pendulum Method

An examination of the authors' data on the coal-in-oil suspension shows that the calculated velocity is sensitive to small changes in the measured periods. This is a disadvantage, since it means that small errors in measurement introduce large errors in the calculated velocity. Obviously the measurement of settling by direct observation in transparent liquids is more accurate than those made with the pendulum. With a few simplifying assumptions one can show that an error in the period ΔT introduces an error in the calculated velocity ΔV given by

$$\Delta V = \frac{2vM'HT_2}{(2qt^2 - ft)\,(T_2^2 - T_1^2)}\Delta T \qquad (5)$$

Here ΔV is measured in centimeters per hour and ΔT in seconds. The term in Equation 5 before ΔT is greater than 1 and plays the role of a magnifying factor. Taking the data on virgin gas oil with 10 per cent coal at $t = 2$ hours and inserting it in Equation 5 gives $\Delta V = 3.97\ \Delta T$. Part of this unfavorable ratio between ΔV and ΔT is due to the large mass, M'. The experiment could have been improved by a reduction in M' through a reduction in the mass of the holder. An aluminum holder would have had a decided advantage.

Expression for Maximum Velocity from Duration of a Constant Period

If the suspension is quite stable, so that there is no change in the period of vibration over a rather long time interval, it is possible to determine the maximum velocity of settling with very little mathematical calculation. In testing samples this offers a rapid method of finding the sample's upper limit velocity. Let V_m be this maximum velocity and t_0 the time interval during which no change in period T_2 is detected. At $t = 0$, Equation 4 becomes

$$(m' + m)x'' = e$$

and at $t = t_0$, it becomes

$$(m' + m)x'' = e + fvt_0 - qv^2t_0^2$$

Since the periods are constants, both left-hand sides of these equations are the same and we have for the maximum velocity

$$V_m = \frac{f}{q} \times \frac{1}{t_0} = \frac{2x_4p'c(p'' - p')}{p''(p' - p) + c^2(p')^2\,(p'' - p')} \times \frac{1}{t_0} \qquad (6)$$

All the quantities in this expression are easily determined and the maximum, V_m, is readily calculated.

Expression for Approximate Velocity of Settling

Often it is desirable to sacrifice accuracy for speed in calculating the settling velocity. The equations for the approximate velocity, V_a, follow from Equations 3 and 4. If h is small, $4\pi^2h^2$ and $8\pi^2h$ may be neglected in Equation 3, and if t is small, qv^2t^2 may be neglected in Equation 4. By combining Equations 3 and 4, we have

$$(m' + m)x'' = \frac{hT_1^2}{T_2^2 - T_1^2}M' + M'y - M''xa = e + fvt$$

Solving for v gives

$$V_a = \frac{M'y - M''xa - e}{ft} + \frac{hT_1^2}{T_2^2 - T_1^2} \times \frac{M'}{ft} \qquad (7)$$

Now let T_{21} be the period (at lower axis) at time t_1, and T_{22} that at time t_2. Inserting these values in Equation 7 and subtracting, we have

$$V_a = \frac{(T_{21}^2 - T_{22}^2)T_1^2}{(T_{22}^2 - T_1^2)\,(T_{21}^2 - T_1^2)} \times \frac{2p''M'h}{(p'' - p')\,p'cx_4^2\,(T_2 - t_1)} \qquad (8)$$

where f has been evaluated and where it has been further assumed that T_1 changes very little during the settling process.

Approximate Velocity Using a Standard Specimen of Known Velocity

When a number of samples are being examined, all of which have the same density and concentration, the above approximate velocity can be expressed in terms of the velocity of any one of the samples, such as a standard sample. This permits a rapid examination and calculation of the velocity for many samples in terms of one standard whose velocity has been determined either by direct observation or by the longer method given above. Let V_s be the velocity of settling for the standard, T_{21s} and T_{22s} the periods of this standard at times t_1 and t_2, V_u the velocity of the unknown, and T_{21u} and T_{22u} its periods. Substituting these quantities in Equation 8 and dividing gives

$$V_u = \frac{(T_{21u}^2 - T_{22u}^2)\,(T_{22s}^2 - T_1^2)\,(T_{21s}^2 - T_1^2)}{(T_{21s}^2 - T_{22s}^2)\,(T_{22u}^2 - T_1^2)\,(T_{21u}^2 - T_1^2)}V_s \qquad (9)$$

Thus with four period readings on the standard and four on the unknown, the velocity of the unknown, V_u, may be readily calculated in terms of the standard, V_s.

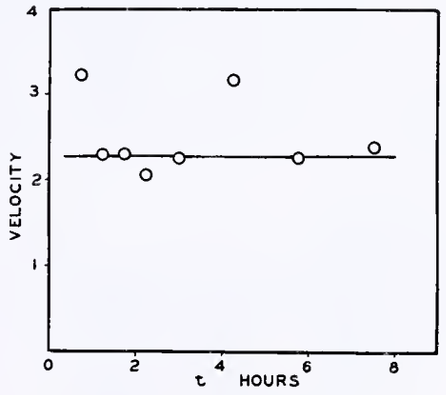

FIGURE 5. CALCULATED VELOCITIES OF PARTICLE FALL AT INTERVALS OF TIME t AFTER SETTLING BEGINS

Conclusion

In the above analysis, the expression for the center of gravity of a settling mixture, which is contained in Equation 3, was an exact one free from assumption as to the mechanism of the settling process. From it the rate at which the center of gravity changes may be calculated. When this expression

was extended to determine the velocity of particle fall, by means of Equation 4, it was necessary to assume that all the particles fell with the same velocity and that this velocity was constant with time. In other words, Stokes' law was assumed to hold and the particles were assumed to have the same diameter.

For those cases where the particles do not fall with uniform velocity, the theory given above must be modified. However, since the method gives an almost instantaneous value for velocity, it can be used to determine just when the mixture failed to settle according to Stokes' law. This suggests that the method could be applied successfully to a study of the interval between the time that settling begins and the time at which "conglomeration" or interference occurs.

In this experiment no special precautions were taken to ensure uniformity of particle size, and consequently there seemed to be no justification for using Stokes' law to calculate particle diameter. It was known that the sizes varied from 1 to 74 microns, but only the 325-mesh screen was available between these sizes. If size distribution could have been determined, it would have been possible to relate this method to other methods of sedimentation. Probably this could best be done by the use of an effective size or uniformity coefficient in place of an average diameter. These terms are used in the analysis of filter sands.

If settling is in the viscous range, interference would presumably be negligible even at high coal concentrations. Stokes' law could then be written as

$$V = \frac{KD^2\rho' - KD^2\rho}{\mu}$$

where K is a constant, D is the effective size, ρ' is the density of the coal, ρ is the apparent density of the liquid, and μ is the apparent viscosity. Both ρ and μ would vary as settling progressed, but for any given system the relation between them could be expressed at least graphically. Since it would be extremely difficult to determine the rate of settling of coal particles in oil by any other method, the samples could not be checked. However, the pendulum method as a method could be tested, using lead shot falling through a transparent liquid and observing the rate of fall visually. A study of settling theory by means of the pendulum is contemplated using these materials.

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Preparing Gas Distributors Using Alundum Disks

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A NUMBER of methods have been described in the literature for the preparation of sintered- or fritted-glass filters from Pyrex glass (*1–8*). These filters are very satisfactory and are especially valuable for use as gas distributors in systems where intimate contact between a gas and a liquid is desired. These methods require considerable time to prepare the glass, and the equipment needed is not always available in chemical laboratories.

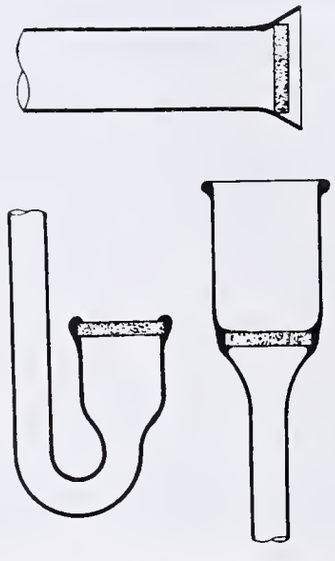

FIGURE 1. SEALING ALUNDUM DISKS TO PYREX GLASS

Attempts have been described to seal Alundum disks directly to Pyrex glass (*2*). While no details have been given regarding the method of preparation of these seals, the results are stated to have been unsatisfactory.

During the last several years, the author has used gas distributors prepared by sealing Alundum disks to Pyrex. These distributors have been very satisfactoy.

A Pyrex tube of slightly smaller diameter than the disk to be used is flared until the disk will fit inside the flared portion as shown in Figure 1 (top). The glass is then heated in the flame of a blast lamp and, as the glass is softened, it is worked against the disk and slightly rolled over the outside of the disk. For use as an aerator, a small connecting tube is sealed on and bent so that the gas is delivered upwards (Figure 1, left).

Alundum thimbles may be used in place of disks.

Filters may be prepared by constricting a Pyrex tube of suitable diameter so that the disk will be held at right angles to the axis of the tube (Figure 1, right). By heating and pulling the tube, a seal is obtained. Filters of this type are convenient for sealing into all-glass systems.

This method requires no special equipment and may be carried out by anyone with an elementary knowledge of glass blowing. Careful annealing of the Alundum-to-glass seal is not required.

Alundum disks are made by the Norton Company, Worcester, Mass., and may be obtained in at least two degrees of porosity. For ordinary laboratory usage, disks 0.75 and 1 inch in diameter are most suitable.

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Autoxidation Measurements on Fatty Oils Using Barcroft-Warburg Apparatus

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THE Barcroft-Warburg apparatus has been widely used in the field of biological chemistry to study autoxidation reactions of various types, including a few cases involving fatty oils, but so far as the authors are aware all measurements have been made at temperatures below 40° to 50° C.

A projected study of antioxidants required a relatively precise and convenient method of measuring autoxidation induction periods by direct oxygen absorption. Because of complexity and lack of flexibility, none of the numerous assemblies previously used seemed satisfactory for the purpose. With the Barcroft-Warburg equipment it was possible to measure induction periods at any temperature from 50° to 110° C., and probably at higher temperatures, with considerable precision. In order to effect oxidation in a satisfactory time interval, most of the determinations have been made at 100° C. in an atmosphere of oxygen.

The equipment permits exact control of experimental conditions, which must be rigidly reproduced if the greatest precision is desired.

Apparatus and Procedure

The Barcroft-Warburg equipment and technique are described by Dixon (*1*). The apparatus, except the flasks, was constructed by the American Instrument Co., Silver Spring, Md., and is shown in Figure 1. The flasks, of special design (Eck & Krebs, New York, N. Y., Catalog No. 210), have volumes of approximately 70 cc. and are fitted with capillary exit valves for gas flushing. Each flask has a central cup about 24 mm. in diameter in which the oil under test is placed. As pointed out by Stephens (*2*), the extent of surface has a pronounced effect on the oxidation rate, and for greatest precision each central cup should have the same dimensions. This is readily achieved by using glass tubing of the same diameter for all reaction flasks. A flask-manometer assembly is shown in Figure 2.

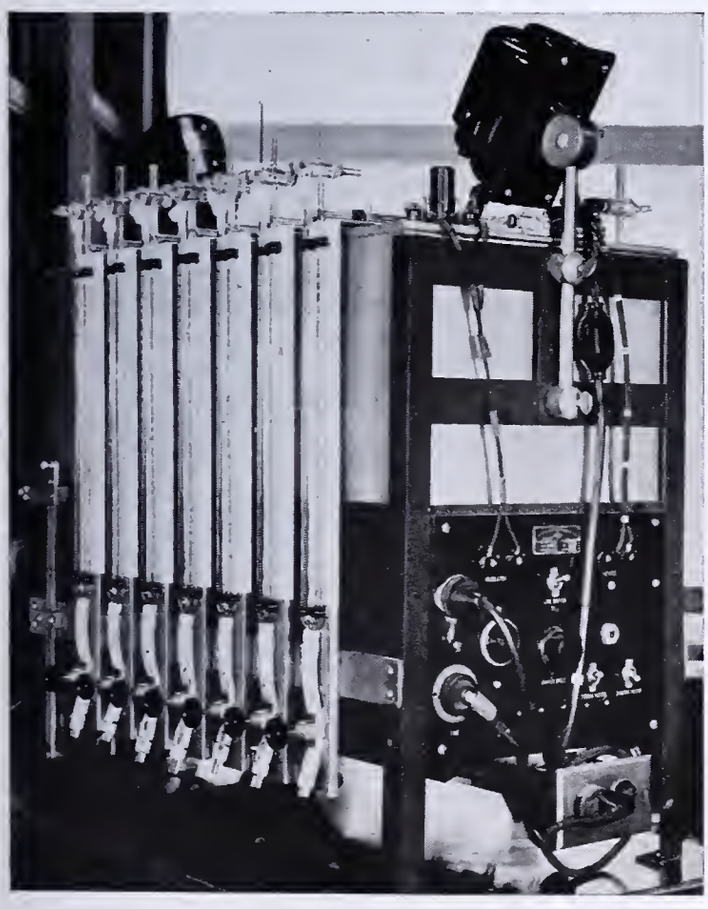

FIGURE 1. BARCROFT-WARBURG APPARATUS (14-MANOMETER MODEL)

Preliminary work indicated that a 0.50-cc. sample of oil is just sufficient to permit completion of the autoxidation induction period when the full scale (30.0 cm. of Brodie solution) is utilized for pressure measurements. The rate of oxygen absorption by 0.50 cc. of oil at 100° C. usually becomes markedly accelerated when the pressure drop amounts to 17 to 21 cm. of Brodie solution, thereby enabling an induction period measurement to be obtained without resetting the manometer. Evidently larger oil samples can be used if manometric resettings are made or denser manometric fluids are employed. A 0.50-cc. oil sample is ideal from the standpoint of gas equilibration, since it forms a thin film about 1 mm. thick which is suitably agitated by the usual shaking conditions of 110 to 120 oscillations per minute at an amplitude of 3 cm.

Temperatures from 50° to 100° C. have been employed, water being used in the bath up to 80° C., and glycerol above 80° C. The temperature control must be precise (at least 0.05° C.). The following procedure has been devised for comparative measurements:

The temperature is set at 100° C., the shaking rate at 110 oscillations per minute, and the amplitude at 3 cm. The shaking rate and the amplitude are sufficient to agitate the oil-gas interface without causing splashing, which would extend the glass-oil interface and prevent the maintenance of reproducible conditions. The determination is made by pipetting or weighing 0.50 cc. of oil into the central cup of a flask, flushing the flask with a rapid stream of cylinder oxygen at a pressure of 10 to 15 cm. of Brodie solution for 15 minutes at room temperature, and then equilibrating at 100° C. for 10 minutes before sealing the system and beginning pressure measurements. The manometers are set at 29 cm. during equilibration. Depending on the rate of absorption, pressure readings are made at various intervals, usually 5 to 30 minutes apart, until about 1500 cu. mm. of oxygen have been absorbed.

Cylinder oxygen is used without purification. Experiments have shown that it is sufficiently dry and provides a reproducible atmosphere.

The flasks must be scrupulously clean. Most of the oxidized fat is removed by rinsing the flasks with carbon tetrachloride, alcohol, and hot water. They are then immersed in cleaning solution for at least 5 hours, rinsed thoroughly with hot water, steamed out, and dried at 110° C.

TABLE I. RATE OF OXYGEN ABSORPTION BY SESAME OIL

Time	Pressure Readings		Volume of Oxygen Absorbed	
	A	*B*	*A*	*B*
Min.	*Mm. of Brodie Solution*		*Cu. Mm.*	*Cu. Mm.*
30	− 3.1	− 3.3	15	17
60	− 5.9	− 7.0	28	36
90	− 14.9	− 15.4	72	78
120	− 20.1	− 21.3	96	108
150	− 29.5	− 30.2	142	154
180	− 35.9	− 38.2	173	194
210	− 46.1	− 46.6	222	237
245	− 56.9	− 58.4	274	297
270	− 68.1	− 68.4	328	348
300	− 80.7	− 83.0	389	423
330	− 97.1	−102.0	468	519
360	−126.9	−143.9	611	733
375	−161.6	−207.7	779	1058
385	−202.9	−272.4	978	1387
390	−232.4	−323.8	1120	1649
400	−326.7	...	1574	....

Induction period, minutes: *A*, 391; *B*, 378

Table II. Reproducibility of Oxidation Measurements on Sesame Oil

Determination Number	Induction Period *Min.*	Deviation from Mean *Min.*
1	420	0
2	422	+ 2
3	426	+ 6
4	427	+ 7
5	433	+13
6	427	+ 7
7	416	− 4
8	414	− 6
9	413	− 7
10	417	− 3
11	417	− 3
12	420	− 0
13	418	− 2
14	416	− 4
15	419	− 1

Arithmetic mean, 420 minutes
Average deviation from mean, 4.3 minutes
Standard deviation, 5.5 minutes
Standard error of mean, 1.5 minutes
Probable error of mean, 1.0 minute

The apparatus includes fourteen manometers and flasks, two sets being used as thermobarometers. Thus a maximum of twelve oil samples can be measured at one time, though at 100° C. it is often possible to measure 24 samples per day.

At 100° C. the choice of a lubricant for the ground joints is very important. Celvacene (heavy), a vacuum grease manufactured by the Distillation Products Company, seems to be excellent for the purpose, since suitable controls have shown no appreciable oxygen absorption by the grease as normally exposed at the edges of the ground joints. Its high melting point is also an important property, since the grease does not tend to leak out of the joints.

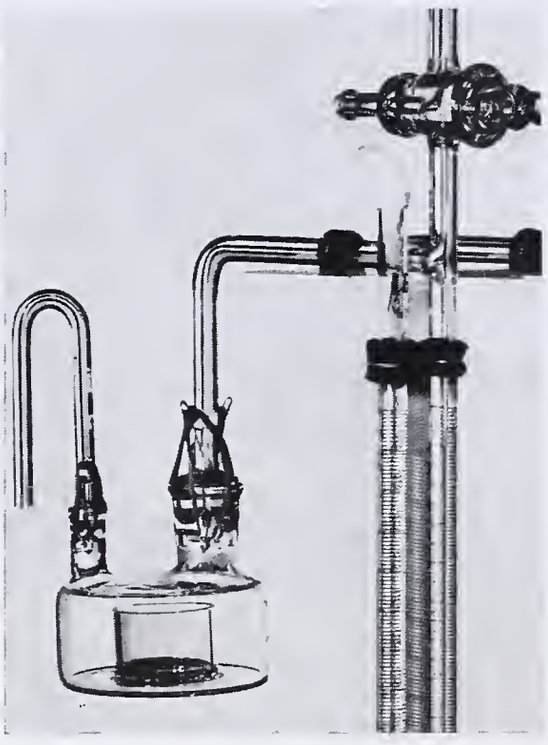

Figure 2. Flask-Manometer Assembly

Discussion of Results

The measurements may be evaluated in the customary manner by converting the pressure readings to cubic millimeters of oxygen absorbed, utilizing calibration data, or the results may be interpreted directly from the pressure measurements. Table I presents the results of duplicate measurements on a refined sesame oil at 100° C. The divergence in volume of oxygen absorbed after the rate becomes accelerated is typical, but this effect is greatly minimized when referred to the time scale.

In Figure 3, results *A* are plotted, showing the rapid acceleration at the end of the induction period. For purposes of comparison the induction period has been taken as the time required for 0.50 cc. of oil to absorb 1000 cu. mm. of oxygen at 100° C. under the experimental conditions previously defined. This choice satisfies, in essentially all cases, the customary definition of the induction period as the time required for the absorption rate to become rapidly accelerated. This procedure also permits one to complete an induction period measurement without resetting the manometers. The time required to reach a maximum absorption rate can be measured by resetting the manometers and making suitable corrections. For other studies it may be best to compare oils on the basis of the time required to reach the maximum rate of oxygen absorption, or possibly to reach any given rate after the acceleration is pronounced.

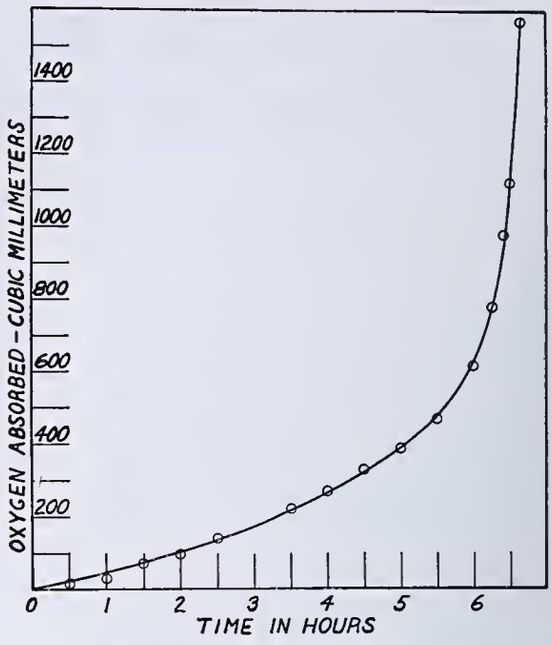

Figure 3. Autoxidation of 0.50 Cc. of Sesame Oil in Oxygen at 100° C.

The time corresponding to an absorption of 1000 cu. mm. may be evaluated by plotting a curve, or it may be obtained by interpolation with adequate precision for general analytical purposes. From a given flask constant the pressure drop corresponding to an absorption of 1000 cu. mm. of oxygen is calculated and then by interpolation of the pressure measurements the induction period is found. Since the flask constants approach 5.0, several pressure readings in the neighborhood of 200 mm. of Brodie solution should be recorded in order to attain the best precision. The measured induction period is corrected for equilibration time by adding 5 minutes. The systems approach temperature equilibrium

Table III. Reproducibility of Oxidation Measurements on Cottonseed Oil

Determination Number	Induction Period *Min.*	Deviation from Mean *Min.*
1	139	+2
2	135	−2
3	142	+5
4	140	+3
5	138	+1
6	145	+8
7	133	−4
8	133	−4
9	135	−2
10	132	−5

Arithmetic mean, 137 minutes
Average deviation from mean, 3.6 minutes
Standard deviation, 4.1 minutes
Standard error of mean, 1.3 minutes
Probable error of mean, 0.9 minute

after 5 minutes but to ensure complete equilibration a 10-minute period is used.

An estimate of the precision of the measurements may be obtained from Table II wherein a series of induction periods measured on the same sesame oil is listed. The determinations were made over a 3-day period using 10 different flasks. It is evident that the precision of a single determination will probably be between ±1 and ±3 per cent. By using the same flask for each measurement and carefully controlling conditions, a precision of 1 per cent is easily realized in measuring sesame oil.

Determinations on a commercial sample of refined corn oil were similar to those with sesame oil with respect to precision and induction period, except that the rate after absorption of 1000 cu. mm. of oxygen was about 50 per cent less. Comparisons might better be made at corresponding rates, but the calculations required are probably not worth while for routine analysis.

Refined cottonseed oil is much less resistant to oxidation than sesame or corn oil as shown by measurements on a typical commercial sample (Table III). Because of the higher oxidation rate, the induction period measurements are not so precise as on the more stable oils.

However, cottonseed oil and other unsaturated oils can be measured with high precision if care is taken to select matched flasks of nearly the same dimensions. The measurements listed in Table IV were made at the same time on six aliquots of a sample of cottonseed oil, using six different flasks and manometers. When the surface factor is thus controlled, the divergence in absorption values noted in Table I is largely eliminated and the induction period is measured with an error of less than one per cent.

Table IV. Oxidation of Cottonseed Oil

Time	Oxygen Absorbed					
	A	*B*	*C*	*D*	*E*	*F*
Min.	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*
5	0.0	0.0	0.0	0.0	0.0	0.0
10	3.6	1.0	1.0	1.0	3.5	2.7
30	30	23	27	24	23	16
60	107	95	84	101	93	79
90	369	360	332	356	353	351
100	739	744	691	716	725	738
105	999	1015	895	974	987	1011
108	1152	1168	1091	1120	1124	1165
100	1280	1292	1212	1243	1268	1278
112	1390	1401	1315	1347	1370	1388
115	1576	....	1486	1526	1560	1599

The tabulated data presented show that the Barcroft-Warburg technique can be extended to elevated temperatures with convenience and relatively good precision.

The accelerated autoxidation of fats and oils and many other substances may, therefore, be studied under reproducible conditions with standard and readily available equipment.

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Presented before the Division of Agricultural and Food Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

An Improved Automatic Continuous Percolator

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THE investigation of plant materials for possible insecticidal properties has necessitated the construction of apparatus suitable for the percolation of the ground plants in quantities ranging from several hundred grams to several kilograms. A simple apparatus (*1*) meeting these requirements has been described and used successfully for some time in this laboratory. A number of improvements have now been made, and the redesigned extractor is shown in Figure 1, diagram I.

Like the previously described extractor, this apparatus is comparatively simple and inexpensive, the condensers and three-necked flasks being standard laboratory equipment. In the old extractor the bottom of a bottle was cracked off to make the percolator, while in the new one a hole was drilled through the bottom of a bottle. It is thus possible to reduce the loss of solvent to a minimum, since the new apparatus is open to the atmosphere only through condenser *I*.

Another improvement has been made in the arrangement of the overflow tube, *B*, which is now slipped over the outlet tube and allowed to rest on stopper *P*, so that excess solvent is returned to the flask through tube *O*. In the old apparatus the rates of distillation and percolation had to be so regulated that the solvent distilled into the percolator faster than the percolate ran into the flask; consequently there always was a return of some solvent to the flask through the overflow tube. If the source of heat happened to be discontinued for some time, the entire percolate would drain down into the flask. The adjustment of the new apparatus is not so critical, particularly if the material to be extracted has not been ground too fine, in which case the only adjustment necessary is to leave stopcock *D* closed.

The percolator, *A*, was made by drilling a hole through the bottom of a bottle. Bottles varying from the common 2.5-liter acid bottles to 5-gallon (18.9-liter) water bottles have been used. In the smaller bottles the hole was placed in the center of the bottom, while in the larger bottles it was near the side. The three-necked flask, *F*, ranged in capacity from 1 to 5 liters; for a 5-gallon bottle it should be 5 liters or larger. The smaller percolator bottles, up to about 5 or 6 liters capacity, may be supported by a sturdy clamp at the neck, but larger bottles, such as the 5-gallon size, should be supported more firmly. An excellent support for the larger bottles is a small table with a hole cut through the top, through which the neck of the bottle is inserted, diagram IV. The table should be of the same diameter as the bottle or slightly smaller, so that tubes *O* and *G* can be brought close to the sides of the bottles for the sake of compactness.

The hole was drilled through the glass bottle with the simple tool shown in diagram III. It was made by wrapping a sheet of galvanized iron 0.8 mm. ($^1/_{32}$ inch) thick and 3.8 cm. (1.5 inches) wide around a cylindrical wooden block about 2.5 cm. (1 inch) thick, through the center of which a metal rod was bolted, the iron sheet being fastened to the block with screws. The tool should be about 0.64 cm. (0.25 inch) smaller in diameter than the hole desired. The hole was cut through the bottom of the securely held bottle by alternately applying and releasing pressure on the tool, which was held in a drill press, with care that not too much heat was produced during the drilling. The cutting edge of the tool was kept well lubricated with a slurry of No. 320 Carborundum powder and water. Owing to the unevenness of most bottles the drill will come through at one place

first. The pressure applied from then on should not be too great. A hole to accommodate a No. 10 stopper was found most convenient for the smaller bottles, and a No. 14 stopper for the 5-gallon bottle.

The glass blowing can be done by anyone having a moderate degree of skill. Instead of the No. $^{12}/_{5}$ ball joint at *C*, a rubber connection may be used, as shown in diagram II, with the rubber tubing wired in place. Stopper *R* should be placed on the tube before bends *S* and *T* are made. Rubber or neoprene stoppers which fit well into the neck of the flask and the other openings have been used satisfactorily, in spite of considerable swelling with such solvents as petroleum ether. As a precaution stopper *P* should be wired in place. Stopper *K* should not be inserted too far into the bottle; if the bottom of the stopper extends more than about 0.3 cm. (0.125 inch), there may be difficulty in removing it at the end of the extraction. The bottom of the stopper should be sliced off as much as necessary. After exposure to the air for a day the stoppers return to their normal size. The steam coil, *E*, was made of glass, but metal pipe may be used where the extract is not of interest or is not harmed by contact with hot metal.

The percolator is filled and operated as follows:

A is supported by a sturdy clamp at the neck. Tube *O*, flask *F*, and steam coil *E* are then put in place. For compactness *O* should be placed against the side of *A*, like the siphon tube of a Soxhlet extractor. [For the sake of clarity in the diagram, it has not been illustrated this way.] *F* can be conveniently supported on a cork ring on the table top. The overflow tube, *B*, is slipped over the outlet tube and allowed to rest on stopper *P*. The top of *B* should be 1.88 to 2.5 cm. (0.75 to 1 inch) higher than the level of bend *N*. Cotton is packed loosely around the overflow tube in the neck of the bottle. A small, very loose plug of cotton may be placed at the top of tube *B* to keep floating particles from flowing into it, but care should be taken not to obstruct the overflow of solvent into this tube.

Stopcock *D* is closed, the percolator bottle is filled about half full with solvent, and the material to be extracted is put into the bottle and allowed to settle evenly by falling through the solvent. The percolator bottle should not be filled above the level of *N* with the material to be extracted. Usually the percolate will be clear, unless the material to be extracted has been ground very fine. In this case the stopcock may be turned to allow the percolate to run out through side arm *Q* into a beaker until it is clear. It is then shut off and the turbid liquid is poured back into the top of the bottle. Enough solvent is poured into the three-necked flask to keep it from half to three-quarters full during operation. The rest of the apparatus, tubes *G* and *J* and condensers *H* and *I*, is then put in place. The opening of *H* was reamed to receive the stopper on *G*. *J* has a small hole at *L* to keep the pressure at the top of the percolator bottle equal to that at the condenser outlets. The side arm of *J* is attached to *M* by a piece of rubber tubing, to prevent siphoning around *O*. The diameter of the glass tubing used will vary with the size of the apparatus. The dimensions shown in diagram I are for a 1-gallon percolator bottle and a 2-liter three-necked flask. Tube *G* should be as wide as will fit into the neck of the flask and should be beveled sharply at the lower end.

A very slow stream of steam is sufficient to boil ether or petroleum ether vigorously. The vapors ascend *G* and are condensed in *H* and *I*, from which the fresh solvent flows into the top of the percolator bottle. *F* may be heated externally by a water, steam, or oil bath if desired, in which case the steam coil, *E*, may be omitted. However, internal heating with a steam coil has proved to be far more convenient than any form of external heating. The arrangement of condensers as shown is extremely efficient, and *H* will condense practically all the vapors. Care should be taken not to boil the solvent so vigorously that all the vapors cannot be condensed in the two condensers. In the summer ice water may be circulated through the condensers by means of a circulating pump, in which case *I* should be protected by a calcium chloride tube.

The adjustment of stopcock *D* depends on how fine the material to be extracted has been ground. With coarsely ground materials *D* may be left shut completely, but with finely ground materials it may be left open. In any case the stopcock should not be left open so much that the solvent level in the percolator bottle drops below the level of *N*. If the source of steam, or other heat, is likely to be interrupted during the night when no one can take care of the apparatus, *D* should be left closed. In normal operation the solvent level in the percolator bottle will always be kept between the level of *N* and the top of *B*. When the material to be extracted has been ground very fine, the supernatant solvent may at first be slightly murky from suspended

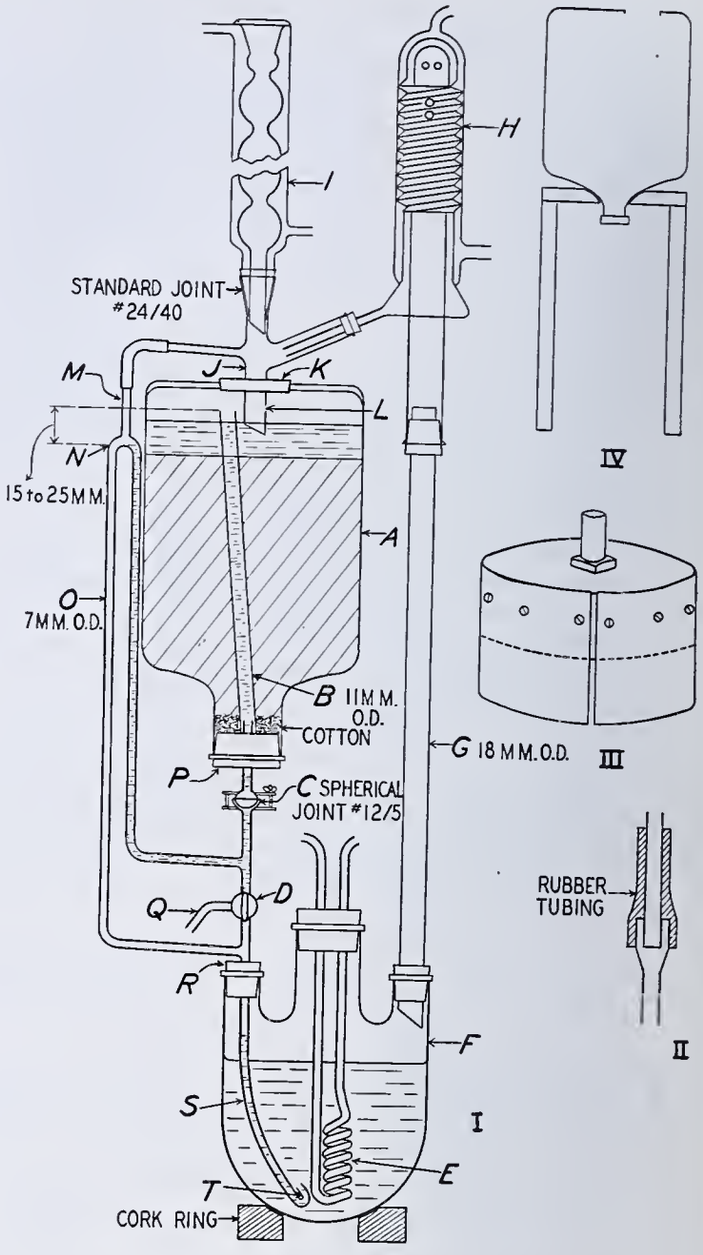

FIGURE 1. AUTOMATIC CONTINUOUS PERCOLATOR

particles. The settling of these particles may be hastened by opening stopcock *D* temporarily to allow the level of the solvent in the percolator to drop a little below the surface of the material to be extracted and shutting off the steam temporarily, if necessary.

In order not to subject the major portion of the extractive to prolonged heating, it is advisable after several hours to withdraw the percolate from flask *E* and replace it by fresh solvent.

When the extraction is completed, most of the solvent may be recovered directly from the apparatus by removing the outlet of *H* from *J* and rotating the condenser so that the solvent can be received in a suitable container. If the condenser water is warm, the distillate from *H* may have to be passed through an auxiliary condenser to make sure that all the vapors have been condensed.

This type of apparatus has been used to extract large amounts of plant material and has worked satisfactorily in every case with very little loss of solvent and very little attention after the apparatus has been adjusted.

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A Powder Compactor for Air-Permeation Experiments

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IN EXPERIMENTS involving the permeability of granular beds, a prime requisite is uniform compaction of the sample. In cases where the permeating fluid is air or some other gas, the compaction methods that have been used are of two main types, vibration and compression.

TABLE I. AIR-PERMEATION RESULTS IN DUPLICATE

(Surface mean diameters in microns taken at random from routine determinations, to indicate degree of reproducibility)

First Run	Second Run	First Run	Second Run
<1	<1	6.9	6.9
1.1	1.1	16	14
1.8	1.8	16	16
2.1	1.9	18	17
3.2	3.2	31	31

The vibration type of method is the more common. The procedure is to place the sample in the cylinder in which it is to remain during the permeation experiment, and either shake or tap the cylinder, laterally or vertically, by hand or by machine, until any channels or pockets in the sample have disappeared and the bulk volume has reached equilibrium. Vibrational compaction was used by Dalla Valle (*1*) for coarse sand; by Traxler and Baum (*4*) for slate, silica, and other materials in pulverized form; and by Gooden and Smith (*2*) for silica powder and other similarly fine materials. This general method is satisfactory for free-flowing samples.

Compression methods are sometimes used alone and sometimes supplemented by vibration. The compacted mass is either formed in the dry state (as is preferable) or formed wet and then dried before use. Traxler and Baum (*4*) experimented with wet-formed briquets for studies of average pore diameter, but found them unsatisfactory because of the tendency to cracking in the process of desiccation. Lea and Nurse (*3*), in determining specific surface of portland cement, consolidated the powder by tapping and then compressed it to a predetermined volume.

In the use of the self-calculating air-permeation apparatus for determination of average particle diameter, described by Gooden and Smith (*2*), it was found that the vibrator originally constituting an integral part of the apparatus was ineffective for certain low-density materials of extreme fineness—that is, around 2 or 3 microns in surface mean diameter. The compression method of Lea and Nurse was also inapplicable here. For reasons involving the wide variety of samples and the automatic calculating feature of the instrument, there is required a column of sample considerably taller than wide, as used, for instance, by Traxler and Baum, rather than the broad, low bed described by Lea and Nurse. The result is that pressure applied to the top of the sample is not transmitted freely all the way to the bottom.

The necessity for a new method of compaction gave rise to the designing of the simple device here described, which works well for all the many types of powders for which it has been tried.

Construction and Use

The compactor, as shown in Figure 1, *a*, is a plunger consisting of two parts, a rod and a foot. The rod is a piece of stiff wire between 1 and 2 mm. in diameter, with kinks distributed throughout its length except for a space of several centimeters near the top reserved as a handle. To the bottom end of the rod is attached the foot, which is shown in detail in Figure 1, *b*, *c*. The foot is of rubber, in the general shape of an inverted stopper with a portion (less than half) cut away on one side. The bottom of the foot (before removal of the segment) must fit smoothly inside the sample tube. If regular rubber stoppers are available in an appropriate size, the required cone frustum may be formed from a stopper by cutting off the large end at the level having the required diameter. Otherwise a piece may be cut out with a crank-type cork borer; a little practice may be required to obtain a suitable taper. The method of joining the two parts of the plunger is to pierce the foot about three fourths through without removing any of the rubber, and force the rod into the pore thus formed.

In use, the compactor is inserted in the empty sample tube, to the top of which a funnel is connected as closely as possible by a short piece of rubber tubing. The funnel stem and the sample tube should have practically the same inside diameter. The sample is poured in on top of the compactor foot. Then the plunger is alternately raised and lowered in short strokes and meanwhile twirled slowly between thumb and forefinger, so that the powder gradually works down past the foot, the latter packing the bed as fast as it is formed. Firm but gentle pressure is applied on each downward stroke. The kinks in the rod assist in feeding the powder and guard against clogging. The twirling motion serves both to increase the effectiveness of the kinks and to prevent compactional inequality due to the failure of the foot to cover the whole cross section. When the bulk of the sample has been packed in place, the plunger is used as a policeman to scrape down any powder left clinging to the wall of the tube.

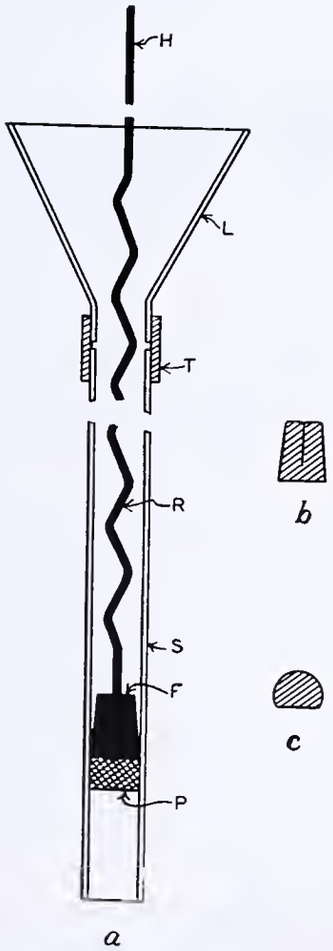

FIGURE 1. COMPACTOR

a. Compactor in sample tube, in position for loading
P. Porous support for sample
F. Foot of compactor
S. Sample tube
R. Rod of compactor
T. Rubber tubing
L. Loading funnel
H. Handle
b. Foot in longitudinal section
c. Foot in cross section

Tests

Only moderate care is required to produce practical uniformity of porosity, as judged by reproducibility of permeation results (Table I).

Comparison of results by this compaction method with those by vibration, as expressed in the respective values obtained for surface mean diameter by air permeation, is afforded by Figure 2. The six samples represent four materials—talc, diatomaceous earth, size fractions of commercial white arsenic, and a coarse fraction of synthetic cryolite. These samples were not in general subjected to size measurements by other methods than air permeation, and in some cases the existence of extraordinary shape characteristics would raise complications

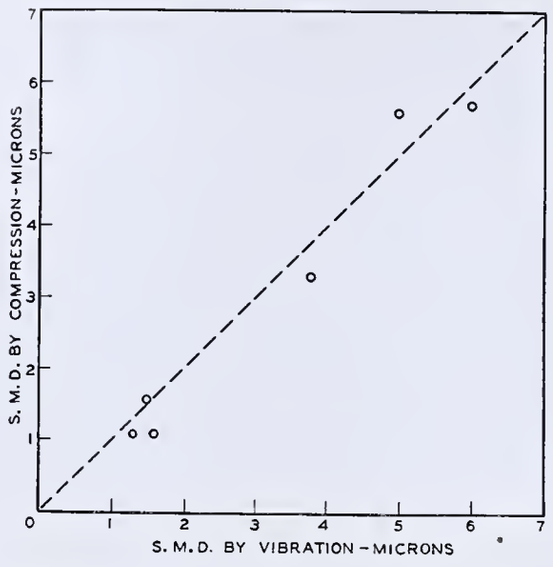

FIGURE 2. GRAPH FOR COMPARING RESULTS OF VIBRATIONAL COMPACTION AND COMPACTION BY COMPRESSION DEVICE

as to the definition of surface mean diameter. The purpose of presenting the comparison is to show that where both methods are usable, substantially the same results are obtained by the new and more convenient method as by the old. Materials coarser than those in the range of the graph usually offer little difficulty of compaction by any method, and many samples of the order of the finest samples here indicated cannot be compacted satisfactorily by any ordinary means of vibration. The system here described not only fills a special need but is suitable for very general use.

Summary

A new device for compacting powders builds up within the sample tube a column of any desired height, the compacting process proceeding from bottom to top concurrently with the deposition of the material. The combined process of loading and packing involves little more work than the simple task of loading alone. Designed particularly for use with the self-calculating air-permeation apparatus for measuring surface mean diameter of powders, it gives promise of equal usefulness in other fields involving permeability of powder beds to gases.

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Photoelectric Colorimetric Technique for the Dithizone System

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A SIMPLE and accurate method for the quantitative determination of lead and other metals with dithizone, which may be used with equipment available in most laboratories, involves the relative absorption of light in different spectral regions and can be readily generalized to other systems.

Since the introduction of the dithizone system of analysis by Fischer (*2*) and its application to the determination of lead by Fischer and Leopoldi (*3*), numerous modifications have been proposed. The current methods may be classified as extractive-titrimetric and as colorimetric. The titrimetric principle was applied to the determination of silver and other metals by Fischer (*4*), to lead by Wilkins and Willoughby *et al.* (*8*), and to mercury by Winkler (*9*). These methods involve the extraction of the metal from the original digest in the presence of fixation complexes when necessary, reliberation of the metal into an acid solution, and titration with a standard dithizone solution. A modification of this technique was introduced by Horwitt and Cowgill (*5*), who extract the lead with excess dithizone, remove the excess dithizone with a cyanide solution, and free the dithizone from the lead dithizonate with an acid solution. The liberated dithizone is then titrated with a standard lead solution.

The modifications of the colorimetric technique introduced by Fischer and Leopoldi (*3*) have been of two types: the "one-color" methods in which the excess dithizone must be removed; and the "mixed-color" method, proposed by Clifford and Wichmann (*1*), in which it is not necessary to remove the excess dithizone. The dithizone is allowed to partition between the aqueous and solvent phases and so modifies the color of the extracted dithizonate according to the relative amounts of dithizone and metal dithizonate. This method has the advantage of not requiring the removal of the excess reagent but has the disadvantage of requiring a series of standard dithizone solutions for the various lead ranges and careful control of the volume relationships between the solvent and aqueous fractions. This procedure is time-consuming if lead determinations are made at irregular intervals, since dithizone is relatively unstable.

The adaptation of the two-color methods to photometric measurements by the construction of standard curves avoids the repeated preparation of standards but has the disadvantage of requiring standard dithizone solutions for the extraction of the metal.

Spectrophotometric methods are selective and permit the determination of each of a series of colored constituents of a mixture. Weigert (*7*), who originated this technique, has determined as many as four constituents in a solution. This method, however, requires an instrument with a monochromatic light which is not generally available. A method adapted to photoelectric colorimeters and which does not require the removal of the excess dithizone or a standard solution of the reagent with which to extract the metal should have a distinct advantage for routine work. With this proposed technique, dithizone of any concentration (approximately 10 mg. per 100 ml. of carbon tetrachloride), but sufficient to ensure complete extraction of the metal from the solution, can be employed. The extract can then be diluted to a definite volume and the necessary photoelectric colorimeter readings taken.

The amount of lead present in the specimen can be determined from a nomograph adapted to the particular photoelectric colorimeter available.

For this work the Evelyn photoelectric colorimeter was employed. The spectrophotometer used for the absorption spectrum studies was described by James and Birge (*6*). A ribbon filament lamp served as the source of light. The slit was kept at a

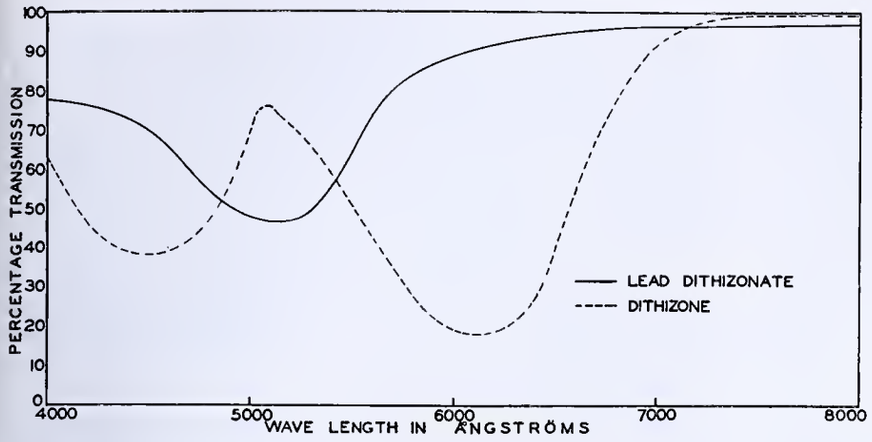

FIGURE 1. TRANSMISSION OF LIGHT

minimum and varied from almost completely closed to approximately 1 mm., depending upon the amount of light energy. The width of the light band was approximately 30 Å. The thickness of the absorption cell was 1 cm.

Principle of Technique

The percentage transmission of light of different wave lengths through solutions of pure lead dithizonate and of pure dithizone is plotted in Figure 1. It will be noted that lead dithizonate (1 microgram of lead per milliliter) in carbon tetrachloride has a maximum absorption in the region of 5200 Å. (45 per cent transmission) and that the absorption of dithizone in carbon tetrachloride is at a minimum in this region. Consequently, a maximum sensitivity to slight changes in the concentration of lead is obtained. The region centered at 6100 Å. is the most sensitive to slight changes in the concentration of dithizone. However, a filter centered at 6600 Å. was selected for this work because the readings in this region are not significantly affected by changes in the concentration of lead dithizonate. The transmission of light through lead dithizonate in this region is approximately 95 per cent.

Monochromatic light of a given wave length passing through a solution containing a mixture of dithizone and lead dithizonate should obey Beer's law, which is expressed by the relationship

$$\log_{10}\frac{\text{intensity of transmitted light}}{\text{intensity of incident light}} = k_d C_d + k_1 C_1$$

where C_d and C_1 are the concentrations of dithizone and of lead dithizonate, respectively, in any convenient unit. The k_d and k_1 are constants for any particular wave length which could be determined from the transmission coefficients of Figure 1. From this equation it follows that when the $\log_{10}$ of the 5200 Å. transmitted light is plotted against the $\log_{10}$ of the 6600 Å. transmitted light for a solution containing a particular value of C_1 and different amounts of excess dithizone, as in Figure 2, the curve should be a straight line. Furthermore, the straight lines for equal increments of C_1 should be equally spaced. However, slight deviations from this rule are observed, since Beer's law is not strictly applicable to conditions of a white light source passing through filters such as are used in photoelectric colorimeters. Other deviations from Beer's law are to be expected at high concentrations because of the effect of solvation. These deviations necessitate the calibration of the apparatus but do not decrease the accuracy of the determination.

The instrument can be calibrated by the preparation of a series of 3 to 5 standard lead dithizonate solutions for each increment of lead. Each successive member of this series must contain an increased amount of excess dithizone. The calibration can also be made with a single standard of lead dithizonate for each increment of lead and the excess dithizone added by 0.1-ml. quantities. The colorimeter readings are taken through the 5200 Å. and 6600 Å. filters on the initial solution and after each addition of the dithizone. The corrections of the 5200 Å. readings for the dilution of the lead dithizonate by the additions of the dithizone would be made as follows:

Owing to the additions of excess dithizone, the volume of the solution is no longer 10 ml. and the concentration of lead is changed correspondingly. L_2, the logarithm of the 5200 Å. reading, must be corrected to be on the basis of an even number (original concentration) of micrograms of lead per 25 ml. of the original solution. To do this we recognize that to a first approximation (corresponding to the assumption of Beer's law), the change in L_2 with the concentration of lead is a constant, k.

$$\frac{\partial L_2}{\partial C_1} = k$$

This derivative can be taken either at constant concentration of excess dithizone or at constant L_1 (logarithm of the 6600 Å. reading), since the lead dithizonate does not absorb the light significantly at this wave length. To evaluate k, it is only necessary to take two concentrations of lead, $(C_1)_a$ and $(C_1)_b$, which have essentially the same values for L_1.

Then

$$k = \frac{(L_2)_a - (L_2)_b}{(C_1)_a - (C_1)_b} \qquad (1)$$

If we know the value of L_2 for a particular concentration of lead, C_1, and we wish to know it for a concentration $C_1 + \Delta C_1$, where ΔC_1 is not too large, it suffices to use the first two terms in Taylor's expansion of L_2.

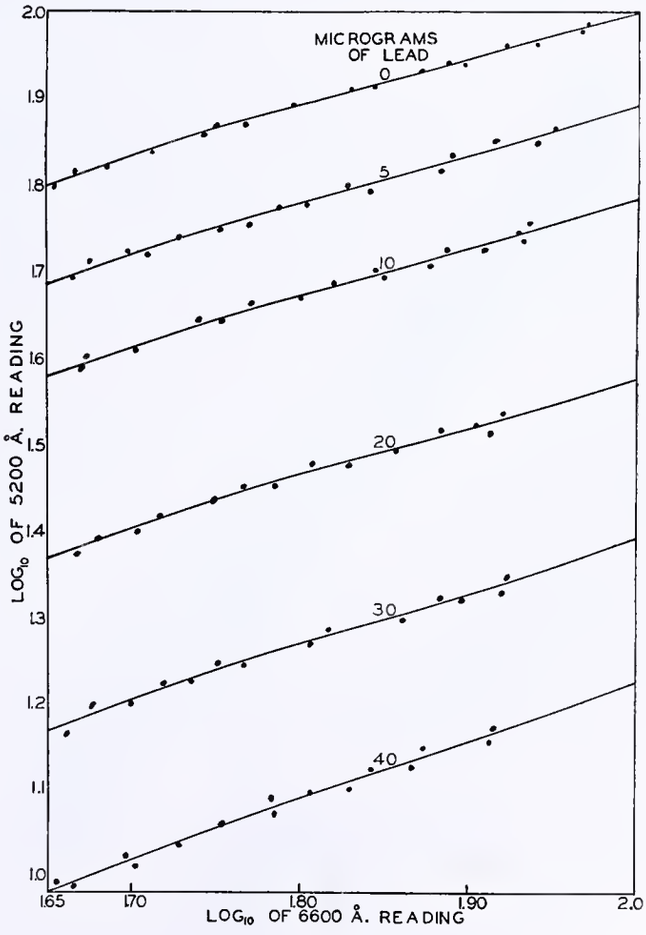

FIGURE 2

$$L_2(C_1+\Delta C_1) = L_2(C_1) + \frac{\partial L_2}{\partial C_1}\Delta C_1 + \ldots = L_2(C_1) + k\,\Delta C_1 \quad (2)$$

The value of k is determined by substitution in Equation 1: Taking two solutions, a and b, with essentially the same L_1,

a $L_1 = 910$ $L_2 = 1.735$ $C_a = 10$ micrograms
b $L_1 = 910$ $L_2 = 1.825$ $C_b = 5$ micrograms

Then $$k = \frac{1.735 - 1.825}{10 - 5} = -0.018$$

L_2 is corrected by substitution in Equation 2: The concentration, C, after the addition of 1 ml. of excess dithizone is $C = 20 \times \frac{10}{11} = 18.2$ micrograms, and L_2 for this concentration is found to be 1.473. Then, L_2 for 20 micrograms = L_2 for 18.2 micrograms + $k(20 - 18.2) = 1.473 - 0.018 \times 1.8 = 1.473 - 0.032 = 1.441$.

The nomograph, Figure 3, was constructed on a basis of concentration of lead per 25 ml. Final dilutions of lead dithizonate of different volumes must be multiplied by the appropriate factor.

Since dilutions of the lead dithizonate have an insignificant effect on the 6600 Å. readings, corrections of L_1, the logarithm of the 6600 Å. readings, are unnecessary.

Construction of Graphs

The standard lead solution containing 10 micrograms of lead per milliliter was prepared from metallic lead 99.98 per cent pure. The extraction of the standard lead solutions was done under conditions similar to those employed for biological specimens. Sulfur dioxide is used on digests of biological material to reduce the iron and oxidize the tin. Other interfering metals are removed chemically because this technique is applicable only to a two-component system. The details of the method for the determination of lead will be described in a subsequent publication.

The desired quantities of lead were extracted from approximately 50 ml. of an aqueous solution of pH 9.5. The solution was prepared by treating about 40 ml. of distilled water with a sufficient amount of sulfur dioxide so that only a small amount (1 ml. or less) of concentrated ammonium hydroxide was necessary to change the indicator (cresol red) completely to the red color after the addition of 5 ml. of 35 per cent sodium citrate and 5 ml. of 5 per cent potassium cyanide. One-half milliliter of concentrated ammonium hydroxide was then added in excess to raise the pH of the solution to approximately 9.5. This solution was extracted several times with pure dithizone to remove lead. The desired quantities of lead were then added and extracted with successive portions of purified dithizone. It is necessary that the dithizone be purified just before it is to be used because oxidized dithizone absorbs light more strongly at 5200 Å. than the purified reagent. The amount of reagent used should be sufficient to ensure complete extraction of the metal; however, the amount of excess dithizone in the extract should be kept at a minimum for the initial readings.

The extraction was completed with two successive portions (approximately 2 ml.) of carbon tetrachloride. The dithizonate was diluted to 25 ml. and filtered through a dry metal-free filter paper, and a 10-ml. aliquot was taken for the readings. Readings were taken through the 5200 Å. and 6600 Å. filters on the initial solution and after each addition of the dithizone. The excess dithizone was added in 0.1-ml. increments. It is essential that the dithizone solution for the additions be washed free of acid to prevent dissociation of the lead dithizonate.

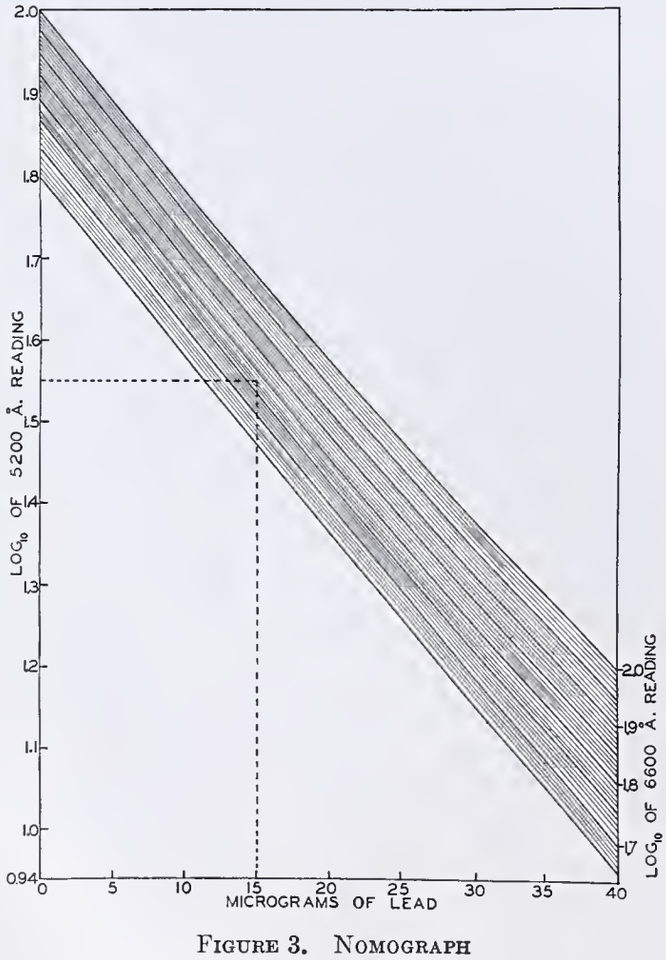

FIGURE 3. NOMOGRAPH

After the logarithms of the 5200 Å. readings had been corrected, the graph (Figure 2) was constructed by plotting these corrected values on the ordinate axis and the logarithms of the 6600 Å. readings on the abscissa axis. This graph should be of sufficient size to permit obtaining the logarithms of the 5200 Å. readings to the third decimal place.

The nomograph (Figure 3) was constructed from data obtained from Figure 2. The logarithms of the 5200 Å. filter readings were plotted as ordinates and micrograms of lead as abscissas. Millimeter paper was found to be the most convenient for this purpose. The most convenient scale was to have 1 cm. on the abscissa axis represent 1 microgram of lead and 1 cm. on the ordinate axis represent a 0.02-variation in the logarithm value. For each quantity of lead indicated in Figure 2 a series of 35 ordinate values (logarithms of the 5200 Å. readings) was obtained. Each reading corresponded to 1 cm. on the abscissa axis where 1 cm. represented a 0.01-variation in the logarithm values of the 6600 Å. readings. The graph was completed by drawing curves through the points which had the same logarithm values for the 6600 Å. readings. From this graph the micrograms of the metal can be read directly after readings have been taken at the two appropriate wave lengths.

Example (Figure 3): If the log of the 5200 Å. reading is 1.550 and the log of the 6600 Å. reading is 1.780, there are 15 micrograms of lead contained in a 25-ml. volume. If the volume of the final extract is 50 ml., the amount of lead in the sample would be twice 15 micrograms, or 30 micrograms.

Summary

A technique adapted to photoelectric colorimeters for the determination of one component in a two-component system is described. The technique is especially adaptable to the dithizone system because it eliminates the necessity of removing the excess dithizone and the use of standard dithizone solutions. It simplifies considerably the procedure for routine analysis. Recoveries of ±1 microgram of lead are obtainable.

Acknowledgments

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A 60-Plate Low-Holdup Laboratory Fractionating Column

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The column described has a packed section of 10.5 feet of tubing 8 mm. in inside diameter, filled with glass helices. An efficiency of about 60 theoretical plates, H. E. T. P. of 2.0 inches, and holdup of less than 0.1 ml. per plate make it useful in the laboratory separation of complex mixtures. The simplicity of the all-glass construction, insulation, and heating elements makes it valuable to the laboratory which must consider ease and economy of construction.

A flexible and effective still head permits operation under total reflux, or variable or total take-off, and at atmospheric or reduced pressure.

IN ATTEMPTING to separate the components of a complex mixture resulting from the hydrogenation of lignin, it became essential to employ a column of high efficiency, low holdup, and moderate cost. Because of the large holdup of columns of the bubble-cap type (*3*), the difficulty of construction and high cost of some types such as the Stedman (*2*), and failure to duplicate the high efficiencies of several columns of the wet-wall type, an experimental study of packings, insulations, operating variables, and over-all efficiencies resulted in the design and construction of a column packed with glass helices, having a flexible head, and a holdup of less than 0.1 ml. per plate. This column has proved itself versatile and successful in the precise analytical fractionation of small quantities of material, and should be of value to any organic laboratory. For a comparison of this column with those previously reported, reference is made to a discussion of laboratory columns (*8*) and a recent review of the literature on laboratory fractionating columns (*14*).

Construction of Column

The principal features of the still are shown in Figure 1.

The glass column was constructed of Pyrex tubing 8 mm. in inside diameter. The packed section was 315 cm. (126 inches, 10.5 feet) long and was attached to a liquid-vapor separator at the bottom, and a still head of the total condensation, variable take-off type. The packing consisted of 210 ml. of single-turn glass helices having an internal diameter of 3 mm. and a fiber diameter of 0.5 mm. (purchased from the American Instrument Co., Silver Spring, Md.).

The liquid-vapor separator attached to the bottom was a bulb 20 mm. in outside diameter having a glass grid sealed in at the equator. The grid supported the packing in the tube and had holes just small enough to prevent passage of the helices. This type of separator was superior to several of conventional design, as well as to that of Snell (*12*), and had a capacity far greater than the flood point of the column, with but slight increase in holdup.

The still head, shown in Figure 2, allowed samples to be taken without breaking the vacuum, except for a short reduction to evacuate the new sample bottle, permitted accurate registration of temperature even at low reflux rates, and provided a visual drip point to determine the rate of reflux. This drip point, as well as the lower drip point (Figure 1), was calibrated by pouring a large, known volume of warm test mixture through the warm wet column and counting the drops. The head, column, and separator were sealed together as one unit with no possibility of leakage at joints. Packing was loaded through the thermometer well. The water condenser served as a heat exchanger, dissipating heat to the atmosphere. Below the condenser was the receiver cup having a capacity of 0.5 ml., and attached to the stopcock by capillary tubing. To ensure complete mixing and sweeping out of early distillate, a small glass funnel was set in the receiver cup. Stopcocks were lubricated with grease or water-base jelly in opposition to the solvent properties of the still mixture.

The insulation consisted of four equal sections of commercial asbestos steam pipe covering, having a 1.87 cm. (0.75-inch) central hole, a 3.75-cm. (1.5 inch) wall and an external diameter of 8.75 cm. (3.5 inches). The glass, packed column was centered in the insulation and supported by corks inserted in the central cavity of the steam pipe insulation. The heater consisted of 300 cm. (10 feet) of No. 26 Nichrome wire in each of the four heating units. It was inserted longitudinally in the steam pipe covering,

2.5 cm. (1 inch) from the outer skin, running parallel to and about 1.25 cm. (0.5 inch) from the surface of the packed tube. The one continuous heater wire was carried through the insulation, brought back in the next quadrant, carried down in the third and back in the fourth quadrant, with the ends brought out through the sides about 2.5 cm. (1 inch) from the end for connection to the line.

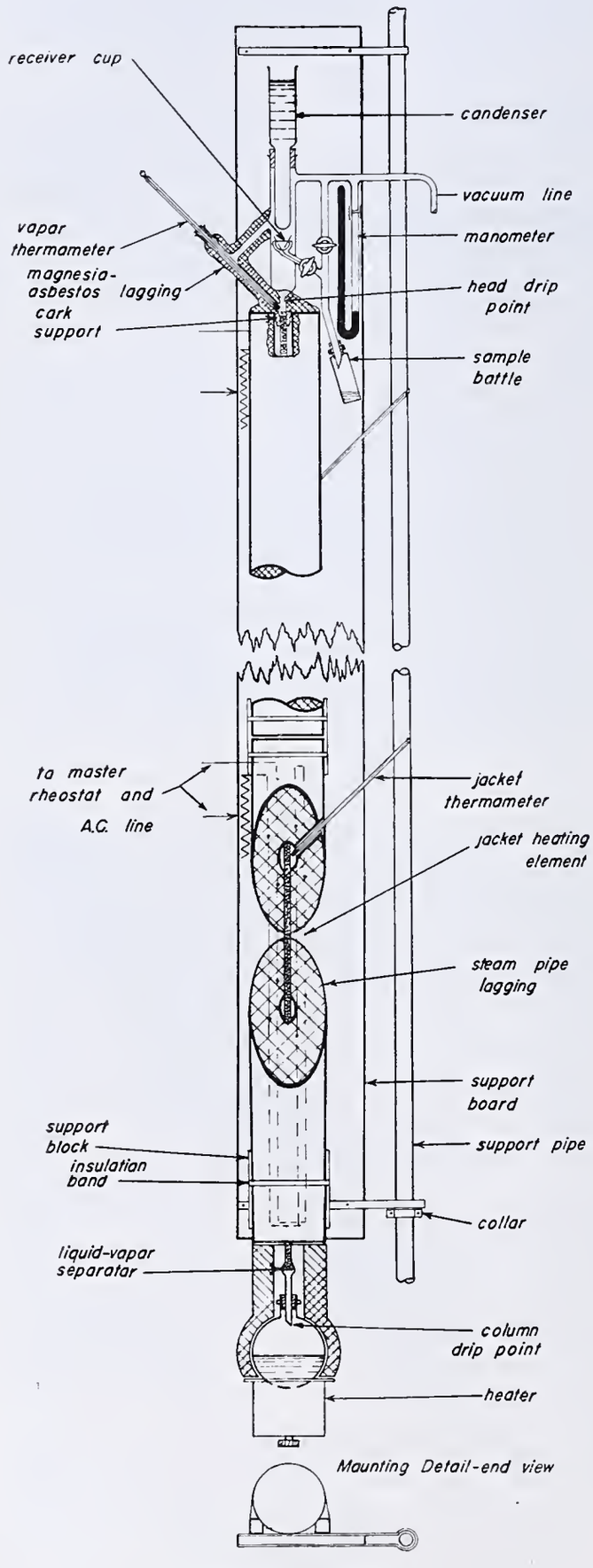

FIGURE 1. ASSEMBLY DRAWING OF COLUMN

A short piece of No. 30 Nichrome wire was attached to one end of each heating element to serve as an individual rheostat, providing an independent heat adjustment for each section of the heater-insulation to maintain a temperature gradient from top to bottom of the column. The four sections of the heater-insulation, each with its individual rheostat, were connected in parallel through a master rheostat to the 110-volt alternating current line to control the temperature of the entire jacket. Preliminary experiments with helically wrapped heating elements showed no advantage over the longitudinal type. Isotherms were determined in an empty section of insulation, with the longitudinal heater installed and ends sealed to eliminate end effect. Temperature variation in any part of the central hole of a section of insulation with the heating element 1.87 cm. (0.75 inch) from the axis and parallel to it in the 3, 6, 9, and 12 o'clock positions (on a cross section) was not more than 0.5° C. In the final, assembled column, thermometers were inserted in each of the four sections to measure jacket temperature, and each section of insulation was protected from heat loss by sealing both ends with asbestos-magnesia mixture. Similar lagging was applied to the glass column projecting below the insulation and to the vapor conduits in the still head.

The heater jacket with the glass column mounted in the center by cork spacer-supports was assembled and attached to a board (2.5 × 15 × 360 cm., 1 × 6 inches × 12 feet, long) by means of standard insulation bands held to the board under wooden cleats which also kept the insulation units aligned with each other. As a final support, a cradle was placed between the board and the still head and the latter fastened to it by a metal strap. The entire board was then mounted on a vertical pipe in the laboratory by two metal U-shaped stirrups at top and bottom, with adjustment for height provided by a metal collar clamped on the pipe. This arrangement permitted lifting and swinging the entire column, replacing the flask with another, and swinging and lowering into its former position over the heater. The cost of all the material used in this column, including lamp-bank rheostats, was less than fifteen dollars.

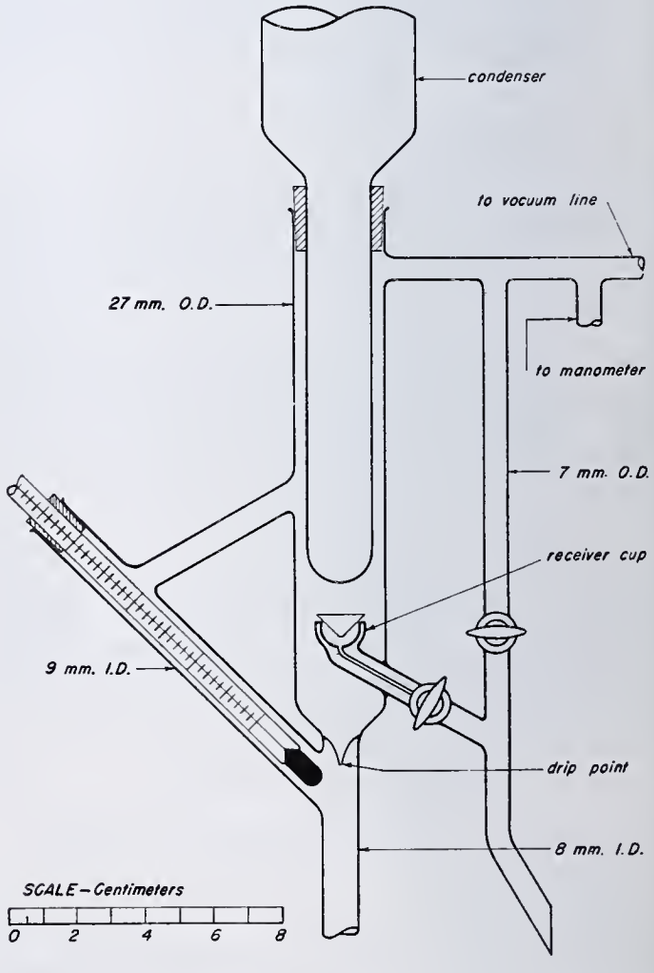

FIGURE 2. TOTAL CONDENSATION, VARIABLE TAKE-OFF STILL HEAD

Operation of Still

The liquid to be distilled was placed in a flask, attached to the column, and protected by an insulating hood, and boiling commenced at a moderate rate. The jacket required several hours to come to temperature and could be adjusted before or after boiling was begun. After reflux from the top of the column was established, the proper temperature of the jacket was attained by the master rheostat and the top-to-bottom gradient adjusted by means of the individual rheostats.

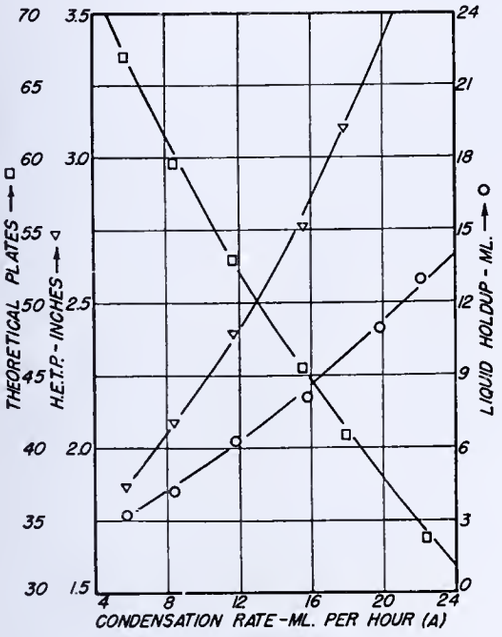

FIGURE 3. OPERATING CHARACTERISTICS OF COLUMN

(A) measured at upper drip point; lower condensation rate approximately constant at 60 ml. per hour. Moderate variations in lower condensation rate from this value had slight effect; variations in the upper condensation rate controlled efficiency.

Data were obtained chiefly on benzene–ethylene chloride mixtures but some values from carbon tetrachloride–benzene. Differences in two mixtures were very small. All tests were made at atmospheric pressure and total reflux. Jacket temperature was adjusted by comparison of upper and lower condensation rates. Take-off was about 1.0 ml. per hour except at 67-plate efficiency when it was 0.5 ml. per hour.

Most efficient fractionation was obtained at condensation rates of 60 ml. or less per hour at the bottom of the column, 10 ml. or less per hour at the top of the column, and with the jacket temperature within 1° to 2° C. of the vapor temperature. Such adiabatic operation could be established in 3 to 4 hours, starting with a cold boiler and column. Attainment of the initial liquid-vapor equilibrium required about 2 to 3 hours on total reflux for a binary mixture with a 3.5° C. separation of boiling points and about 0.5 to 1 hour to re-establish equilibrium conditions after removing a 0.5-ml. sample. The rate of take-off at 60-plate efficiency was about 1 ml. per hour. Better fractionation was obtained at lower condensation rates with the jacket temperature within a degree or so of the vapor temperature. Even at a condensation rate as low as one drop per minute from the still-head drip point, the thermometer recorded the correct temperature with no fluctuation of the mercury column. Correct temperatures were registered at all points on the boiling curve, "breaks" as well as "plateaus". The initial temperature in the still head often dropped 2°, with a binary mixture whose components boiled 3.5° apart, by the time equilibrium was established; on some experimental mixtures, a drop of 20° from the initial temperature was observed. Removing a small sample after equilibrium was attained caused a temperature rise which decreased to a constant value as equilibrium was re-established.

Efficiency Tests

The column was tested, with equipment and operation as described, at atmospheric pressure and with total reflux.

Binary mixtures of carbon tetrachloride–benzene and benzene–ethylene dichloride were first distilled through a 25-plate column, taking only a heart cut which had the correct and constant boiling point and specific gravity. All measurements of composition of the mixtures were by specific gravity. Efficiencies were calculated by the Fenske equation (*6*), the Dodge and Huffman equation (*5*), and the graphic method of McCabe and Thiele (*7*). Better agreement in results was obtained by the McCabe and Thiele method, probably because neither of the test mixtures is ideal. The liquid-vapor equilibria data were taken from the values of Rosanoff and Easley (*9*). All efficiencies reported here were determined by the McCabe and Thiele method and have one plate subtracted for the still pot.

Graphic summaries of operating variables are shown in Figures 3 and 4.

The flood point was approximately 400 ml. per hour. The pressure drop of the column was not determined.

Holdup

The amount of holdup is of great importance in laboratory fractionation. The bubble cap column has a holdup of about 1 ml. per theoretical plate (*3*), the Stedman 0.17 ml. per plate (*2*), the spinning band 0.12 ml. per plate (*1*), the column reported in this paper 0.06 ml. per plate, and the Selker-Burke-Lankelma column 0.04 ml. per plate (*11*).

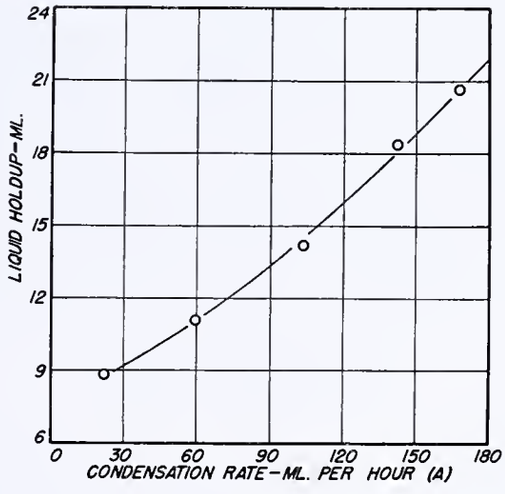

FIGURE 4. LIQUID HOLDUP AS A FUNCTION OF THE RATE OF TOTAL CONDENSATION

(A) measured at lower drip point. Upper condensation rate not accurately controlled but jacket temperature kept within 3° C. of vapor temperature. Data obtained under same conditions as Figure 3.

Holdup was determined with stearic acid by the method of Tongberg, Quiggle, and Fenske (*13*), by the drainage method, and by pouring a known quantity into the top of the warm dry column and measuring the drainable and nondrainable quantities. That reported in Figure 3 is the actual operating holdup (*13*) of the two binary test mixtures. Three factors had an important influence on the quantity of holdup: the surface tension of the mixture, the rate of vaporization (with total reflux), and the temperature of the jacket. Aqueous solutions usually had from two to four times the holdup of organic mixtures. Fortunately, holdup decreased with decreasing rate of vaporization and increasing efficiency.

Furthermore, maintaining the jacket temperature within 1° of the vapor temperature greatly reduced holdup; Selker, Burke, and Lankelma (*11*) also noted a perceptible decrease.

Elimination Curves of Known Systems

In order to show exactly what recovery could be obtained from a known mixture, distillations were carried out when only a small quantity of the more volatile component was present. The final, complete elimination curve gives the shape and magnitude of the boiling curve between plateaus and is a check on the quantity of holdup and a test of the minimum quantity of volatile component which is necessary to yield a pure distillate. The elimination curve is the basis for estimates of quantitative separation of unknown mixtures and a guide to the control of operating variables in such a separation.

Various elimination curves are shown in Figure 5. These data were obtained with the jacket temperature within 1° to 2° C. of the vapor temperature, at about 60-plate efficiency, at total reflux, take-off of 1 ml. per hour, and other conditions substantially as indicated by Figure 3. Figure 4 shows the rate and volume in which the more volatile component was eliminated from a charge of given volume and concentration. All the curves in Figure 4 are really enlargements of the break or boiling curve between two plateaus (representing a pure compound or constant-boiling mixture).

Discussion

A number of interesting efficiencies were experimentally determined during the preliminary fundamental study of analytical columns. The following were determined by benzene–carbon tetrachloride unless otherwise stated.

A single, empty 14-mm. inside diameter tube 150 cm. (5 feet) long with heater jacket, operated under adiabatic conditions at very low reflux rates approaching those used by Rose (*10*), gave an H. E. T. P. (height of equivalent theoretical plate) of 25 cm. (10.0 inches), seeming to indicate that efficiencies of short (30-cm., 12-inch) columns bear no relation to longer columns, presumably due to channeling, now a more or less generally accepted explanation. Using a closed inner tube of 12-mm. outside diameter in the above 14-mm. inside diameter empty tube after the manner of Craig (*4*) gave an H. E. T. P. of 33.75 cm. (13.5 inches). Adding concentric tubes of the diameters and clearances specified by Selker, Burke, and Lankelma (*11*) gave an H. E. T. P. of 15.75 cm. (6.3 inches). Numerous changes were made in the reflux distributor which they recommended on top of the concentric tubes without changing efficiency.

Operating conditions, including vaporization rate, offtake, total reflux, etc., were carried through the entire range specified, but in spite of the most meticulous attention to details it was not possible to assign a cause for failure to duplicate the reported efficiency. The column was in continuous testing operation for about 6 weeks and lack of time made further testing impossible. It may be that differences in wall thickness of the glass caused the failure, since this is reported to have an effect, or that the deviation from ideality of the benzene–carbontetrachloride test mixture did not permit duplication of the ideal mixture used by Selker, Burke, and Lankelma.

Removal of the concentric packing and adding glass helices gave an H. E. T. P. of 5 cm. (2.0 inches). Another column 12.5 cm. (5 feet) long having an inside diameter of 8 mm. instead of 14 mm. and similarly packed with helices also gave an H. E. T. P. of 5 cm. (2.0 inches). This made possible a considerable decrease in the volume of packing, and hence of holdup, and seemed to indicate that irregular packing next to the wall was unimportant or similar in both 14- and 8-mm. tubes and suggests that further reduction in the size of the tube, and hence of holdup, might be achieved. The final 315-cm. (10.5-foot) column was then built as the maximum convenient size and had an H. E. T. P. of 5.25 cm. (2.1 inches). It thus appeared that little difference in channeling existed in the 150-cm. (5-foot) and 315-cm. (10.5-foot) columns. Both had the operating characteristics of the typical wet-wall column described by Rose (*10*) and Craig (*4*)—i. e., increasing efficiency approached zero vaporization asymptotically. In other words, the lower the reflux rate the higher the efficiency, efficiency being limited by ability to maintain a low reflux rate.

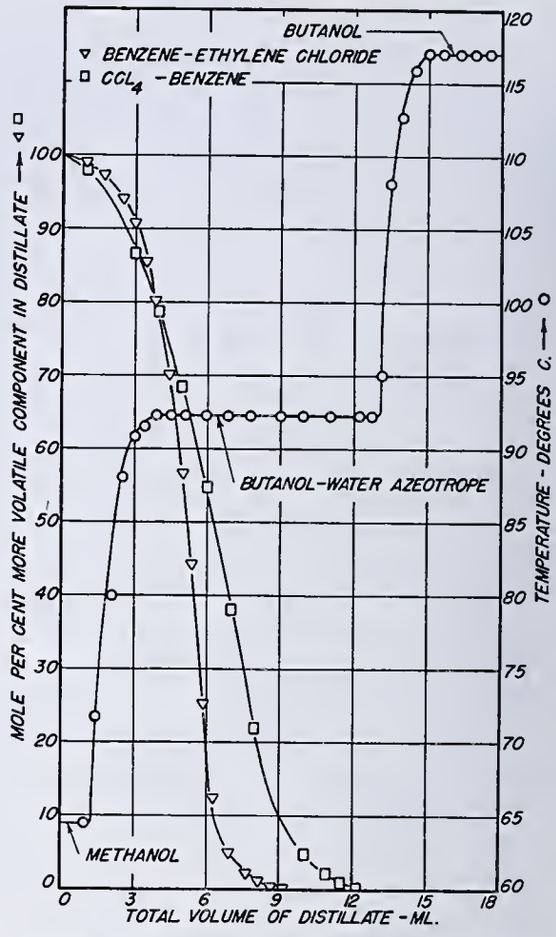

FIGURE 5. ELIMINATION CURVES OF KNOWN MIXTURES IN 60-PLATE COLUMN

Charge for benzene–ethylene chloride and carbon tetrachloride–benzene mixtures was 4.6 grams of more volatile plus 134.0 grams of less volatile component; recovery of volatile component was 99.7 to 99.8 per cent complete. Volume of methanol-water-butanol charge was 1000 ml. Steeper elimination curves were obtained by increasing flask concentration and reducing flask volume.

Voltage variation in the alternating current line used in these tests necessitated laborious manual control and limited the efficiency obtainable.

Other types of packing were also studied in the preliminary tests and although not very efficient are included here for comparison. These efficiencies were determined with benzene–carbon tetrachloride with reflux ratios between 5 to 1 and 10 to 1 unless otherwise noted.

1. Vigreux column with 30-cm. (12-inch) "packed" section and having a double vapor jacket of the Widmer type in addition to external insulation. H. E. T. P. = 11.75 cm. (4.7 inches).
2. Same but with a 107.5-cm. (43-inch) "packed" section. H. E. T. P. = 16.25 cm. (6.5 inches).
3. Raschig rings 7 mm. in diameter and 7 mm. long, packed section 50 cm. (20 inches) long. H. E. T. P. = 10.25 cm. (4.1 inches).
4. Brass jack chain, 100-cm. (40-inch) packed section. H. E. T. P. = 10 cm. (4.0 inches).
5. Glass helices, packed section 150 cm. (60 inches), total reflux, H. E. T. P. = 5 cm. (2.0 inches).

Most of the work on extremely efficient fractionating columns has been done by petroleum chemists who were not handicapped by limited quantities of material. Still-pot charges of at least several liters are commonly reported, and quantities of material between plateaus on the boiling curve of several hundred milliliters are not uncommon. This freedom is reflected in the design of many efficient columns and has prohibited the application of this effective tool to small quantities of material.

Recognition of the great need of efficient columns for the

separation of small quantities of material is shown by the development of 70- to 100-plate columns of extremely small holdup, such as those of Stedman (*2*), Selker, Burke, and Lankelma (*11*), and Baker, Barkenbus, and Roswell (*1*). Since columns of this latter type are still new and not yet widely used, there yet remains to be built up a literature of the specialized and difficult technique of successful operation and the later ingenious stratagems invariably proposed in all types of research to solve specific problems, arriving ultimately at a high level of efficiency, ease of operation, and simplicity, and low cost of apparatus. It would seem that the infrequent use of such columns in the average organic research laboratory is due chiefly to unsuitable design, complexity of operation, and cost. Yet such columns are an extremely powerful tool which can simplify many a formerly involved chemical separation to a clear-cut, rapid, and easy physical separation.

To the end of improving and simplifying operation, several deviations from normal practice were advantageously used in the analytical separation of small quantities. Since less than 1 ml. of a compound was sufficient for determination of the physical constants, this was obtained, even though the quantity available was less than that necessary for obtaining a pure compound under normal operation. Reaction mixtures in this laboratory often amounted to 1 liter or more with only a few grams of a certain product in the mixture.

To illustrate how the separation was effected, suppose 4 grams of benzene are in 1 liter of ethylene dichloride; the concentration is 0.4 per cent or about 0.5 mole per cent. In this dilution, 60 theoretical plates cannot separate pure benzene. However, if 20 grams were distilled, all the benzene would be in it and the concentration would be 24.2 mole per cent; a column of 60 theoretical plates with a sufficiently low holdup can easily separate pure ethylene dichloride from 20 grams of such a 24.2 mole per cent solution.

The column described actually separated pure ethylene dichloride from a solution having a concentration of 5.4 mole per cent.

To avoid the preliminary distillation to concentrate the compound sought, a slightly higher rate of vaporization was established, causing a greater amount of holdup, say 15 ml. The large flask was then removed and a short test tube quickly attached. On slowing down the rate of vaporization, the holdup also dropped to 4 to 5 ml., causing about 10 ml. to drain into the test tube flask.

One particular distillation may be cited as an example of this method. Pure benzene was removed from the top of the column until the distillate showed a trace of ethylene dichloride. The flask composition was a little less than 6 mole per cent benzene. The vaporization rate was increased, with total reflux; a test tube was substituted as a flask; and 8 ml. of holdup were drained back into the flask when reduction in the rate of vaporization permitted the holdup to decrease to 3.5 to 4 ml. This 8 ml. of holdup which returned to the flask had a composition of 78 mole per cent of benzene as compared to the earlier concentration of 6 mole per cent. After obtaining a small sample of the pure compound for determination of the physical constants, the total quantity was determined by the elimination curve.

A variation in the normal elimination curve was obtained by using a nonlinear thermal gradient from top to bottom. For example, in separating small quantities of methanol from a methanol-butanol-water mixture the methanol boiled at 65°, and the water-butanol azeotrope at 92°. Since only about 12 plates were necessary to effect the separation, the jacket temperature in the lower three sections was adjusted to 91°, and in the upper section to 63°. Accordingly, the holdup of methanol was reduced to one fourth of the normal column holdup and not only was more pure methanol obtained but the quantity of the mixture between plateaus correspondingly reduced.

An extremely close approach to adiabatic operation could be made by observation of the condensation rates at top and bottom of the column. The head was designed especially to provide a visual drip point and reflux distributor [although the 5-foot (150-cm.), 8-mm. inside diameter column had the same H. E. T. P. with the reflux running down the walls]. Best efficiencies were obtained when the condensation rates were very low—i. e., 60 ml. per hour at the bottom of the column, and 5 to 10 ml. per hour at the top. They were established by adjusting the heat in the boiler and then the heat in the jacket, and were vastly better guides than the thermometer readings of the jacket temperatures.

As a measure of the efficiency of this type of jacket, the data of Selker, Burke, and Lankelma are of interest. Their 5-foot, silvered vacuum jacket alone, without heater, condensed about 195 ml. per hour at 97-plate efficiency at a reflux rate of 195 ml. per hour plus the take-off rate, 0.16 gram per hour; in other words, the jacket alone condensed 99.9 per cent of the reflux. The jacket alone, described in this paper, operating at condensation rates of 30 to 45 ml. per hour at the bottom and 6 to 12 ml. per hour at the top, condensed only 24 to 33 ml. per hour or 73 to 80 per cent of the reflux and could be adjusted to condense from 0 to 100 per cent of the reflux.

This column is now effectively separating complex hydrogenation reaction mixtures. It is hoped that its utility will not be limited to this laboratory alone. It has given sharp separation of mixtures on which less efficient columns (20 to 25 plates) failed even to show any plateaus in the boiling curve, and hence the location of the boiling points of any compounds. With this degree of mixture, identification of individual compounds is practically hopeless. In common with others who have used efficient columns, the author believes their use is an absolute necessity for precise work and shares the opinion of Bruun and Faulconer (*3*), "It should be particularly noted that with a column containing only 26 plates, the separations to be expected are such that even compounds boiling 12° apart cannot be completely separated. Consequently, the need of columns containing a larger number of plates cannot be emphasized too strongly. Generally speaking, efficient separation of complex mixtures in stills containing less than 30 plates is almost hopeless. Similarly, packed columns with heights below 1.5 meters (5 feet) should be used only for separation of a few of the very simplest known binary mixtures."

Acknowledgment

To the expert skill of Ray G. Newbury, glass blower in the University of Washington, in constructing the more difficult parts of a number of fractionating columns, the author is greatly indebted.

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Determination of Lead in Biological Material

A Mixed Color Dithizone Method

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THE principal interfering elements in the determination of lead in biological material with dithizone are ferric iron, stannous tin, thallous thallium, and bismuth.

Ferric iron has an oxidative effect on the dithizone causing considerable decomposition of the reagent. Stannous tin and thallous thallium combine with dithizone under essentially the same conditions as lead.

To overcome the effect of the ferric iron, Behrens and Taeger (*1*) employed hydroxylamine hydrochloride. This reagent, however, is inadequate for specimens containing appreciable quantities of iron and a preliminary extraction of the lead with excess dithizone is necessary. The lead is then stripped from the lead dithizonate solution with a dilute nitric acid solution and reextracted with purified dithizone.

Since tin can be expected to be present in varying amounts in all biological material upon which lead determinations are made, it is necessary to remove or inactivate it.

Laug (*8*) suggested dry-ashing with nitric acid to change the tin to metastannic acid. Fischer and Leopoldi (*2*) suggested volatilization of the tin as stannic bromide. Wichmann and Clifford (*10*) dissolved the ash with perchloric acid before treating it with hydrobromic acid and bromine in order to facilitate volatilization of the tin as stannic bromide.

While sulfur dioxide (sulfurous acid) was suggested originally by Fischer and Leopoldi (*3*) as a preservative for dithizone, it has not been employed as a reagent for inactivating the ferric iron and stannous tin. Sulfur dioxide will reduce the iron and oxidize the tin according to the following reactions:

$$2Fe^{+++} + SO_3^{--} + H_2O \longrightarrow 2Fe^{++} + SO_4^{--} + 2H^+$$

$$3Sn^{++} + SO_2 + 6H^+ \longrightarrow 3Sn^{++++} + H_2S + 2H_2O$$

The introduction of this reagent has a distinct advantage because it prevents the decomposition of dithizone by the ferric iron regardless of the amount present and at the same time oxidizes any stannous tin which may be present. This reagent eliminates the necessity of a preliminary extraction. In the absence of bismuth the lead can be quantitatively determined with one extraction.

To remove bismuth, Willoughby, Wilkins, and Kraemer (*12*) and Hubbard (*6*) suggested adjusting the solutions containing the lead and the bismuth to pH 2.0 and extracting the bismuth with a large excess of dithizone. Horwitt and Cowgill (*5*) suggested extracting the bismuth at a pH of 3.0 to 3.5. Gant (*4*) eliminated the bismuth by adjusting the stripping acid solution to pH 2.0. The bismuth which partitions into the aqueous phase with the lead is then extracted with excess dithizone.

This separation of the lead from the bismuth was essentially confirmed in this laboratory. It was observed that bismuth is extracted with difficulty with dithizone at pH 2.0; however, very little partitions into the aqueous phase at this pH when present as the dithizonate. A quantitative separation of the lead from the bismuth can be effected by stripping the lead from the dithizonate with a nitric acid solution of pH 2.3 to 2.5. At this pH satisfactory recoveries of both metals can be obtained.

Thallium did not interfere with the determination of lead under the conditions of this procedure. The mechanism by which the thallium is inactivated has not been determined. Apparently the metal is oxidized in the digestion process and is not reduced by the sulfur dioxide.

In the presence of antimony, lead recoveries are low because the two metals form a stable complex during digestion and prevent the lead from combining with dithizone. The character of the complex has not as yet been determined. However, preliminary studies indicate that the lead is combined in the proportion of three atoms of antimony to one of lead when digested in the presence of sulfuric acid. Digestion of the specimen with nitric and perchloric acids alone did not inactivate the lead. In the absence of antimony, a small amount of sulfuric acid is preferred to minimize the hazards of a perchloric acid digestion. Dry-ashing and subsequent solution of the ash with perchloric acid and hydrochloric acid did not inactivate the lead.

Method

REAGENTS AND MATERIALS. Potassium cyanide, 5 per cent solution, C. P. quality. Sodium citrate, 35 per cent solution, C. P. quality. Ammonium hydroxide, concentrated, redistilled. Hydrochloric acid, concentrated, redistilled if necessary.

Dithizone solution, 0.1 mg. per milliliter of redistilled carbon tetrachloride, purified before using. Cresol red indicator, 0.04 per cent solution. Calcium chloride, 10 per cent solution, purified by extraction with dithizone.

Ammonium oxalate, saturated solution. Oxalic acid recrystallized from a 1 to 1 hydrochloric acid solution several times, dissolved in metal-free water, and neutralized with redistilled ammonium hydroxide.

Nitric acid, concentrated, C. P. quality. Sulfuric acid, concentrated, C. P. quality. Sulfur dioxide, obtained in cylinders or generated by treating sodium hydrogen sulfite with concentrated sulfuric acid. Perchloric acid, 60 per cent. Hydrogen peroxide superoxol.

Filter paper, metal-free, Whatman No. 40 extracted with dithizone in carbon tetrachloride and finally with pure carbon tetrachloride to remove the dithizone.

Glassware, cleaned with a nitric acid–hydrochloric mixture

Table I. Logarithms of Readings with 5200 Å. and 6600 Å. Filters for Various Quantities of Lead

(With increasing amounts of excess dithizone)

	Micrograms of Lead					Log10 of 6600 Å. Readings[a]
	0	10	20	30	40	
Log10 of 5200 Å. readings with increasing amounts of excess dithizone	2.000	1.782	1.574	1.376	1.195	2.000
	1.974	1.754	1.542	1.342	1.159	1.950
	1.946	1.724	1.510	1.309	1.124	1.900
	1.920	1.698	1.484	1.282	1.092	1.850
	1.892	1.670	1.458	1.251	1.058	1.800
	1.866	1.644	1.429	1.221	1.022	1.750
	1.832	1.614	1.397	1.187	0.984	1.700
	1.797	1.578	1.360	1.149	0.950	1.650

[a] Each logarithm of 6600 Å. reading corresponds to logarithms of 5200 Å. readings for various quantities of lead.

and rinsed with metal-free water. Previous to using, it is rinsed with dithizone and finally with carbon tetrachloride.

Procedure

Urine. The technique for the separation of lead from urine is essentially the same as that suggested by Ross and Lucas (*9*).

Two hundred milliliters of urine are measured into a 225-ml. Pyrex centrifuge cup and made faintly acid to litmus. Three milliliters of 10 per cent calcium chloride solution are added and the lead and calcium are precipitated by adding 20 ml. of the saturated ammonium oxalate solution. After approximately one hour the specimen is centrifuged and the supernatant fluid carefully decanted. The precipitate is digested with 2 ml. of perchloric acid over a low flame. The digestion is completed by adding hydrogen peroxide dropwise until the solution remains colorless. The digest is then transferred to a 125-ml. separatory funnel. For convenience, the volume of the solution should not exceed 50 ml. Sulfur dioxide is slowly passed through the solution for about 5 minutes, and the solution is permitted to stand for at least one hour.

Twenty milliliters of the 35 per cent sodium citrate, 10 ml. of the 5 per cent potassium cyanide, and 4 to 6 drops of the cresol red indicator are added in the order mentioned. The solution is adjusted to approximately pH 8.4 (indicator completely changed to red color) and extracted with 5-ml. portions of the purified dithizone (shake for about 2 minutes for each extraction) until the carbon tetrachloride phase is no longer discolored by the presence of lead dithizonate. The extraction is completed by washing twice with a few milliliters of pure carbon tetrachloride. The extracts are pooled by draining them into a second separatory funnel. After each extraction it is important to allow a sufficient time for the complete separation of the two phases in order to prevent a transfer of any of the alkaline aqueous solution to the second separatory funnel. Should this occur, it may result in an appreciable loss of lead due to a significant change in the pH of the acid stripping solution. The lead is stripped from the dithizone by shaking for one minute with about 40 ml. of a nitric acid solution, pH 2.3 to 2.5. The dithizone solution is drained off after the two layers have completely separated and the aqueous phase is extracted several times with pure dithizone to remove any bismuth which may be present. A final extraction is made with pure carbon tetrachloride.

The aqueous phase containing the lead is treated with a sufficient amount of sulfur dioxide so that only a small amount of concentrated ammonium hydroxide (1 ml. or less) is necessary to change the indicator (cresol red) completely to the red color after the addition of 5 ml. of sodium citrate and 5 ml. of potassium cyanide. One-half milliliter of concentrated ammonium hydroxide is added in excess to raise the pH of the solution to 9.5. The lead is then extracted with 5-ml. portions of pure dithizone until the carbon tetrachloride layer is no longer discolored by the presence of lead dithizonate. The extraction is completed by washing twice with pure carbon tetrachloride. The extracts are drained into a 25-ml. volumetric flask, diluted to volume, and filtered through a dry metal-free filter paper into a clean colorimeter tube. The readings with the 5200 Å. and 6600 Å. filters are taken and the quantity of lead is determined according to the method described by Kozelka and Kluchesky (*7*).

The data necessary for the construction of the nomogram for an Evelyn colorimeter are given in Table I.

The values for eight points on the ordinate axis with the corresponding 6600 Å. axis values are given for the quantities of lead indicated in the table. After the curves through the points with the same 6600 Å. values have been drawn, the spaces between these curves are subdivided into five equal parts. Each subdivision represents a 0.01 variation in the 6600 Å. value. Satisfactory recoveries for various quantities of lead have been obtained with a similar graph with three different Evelyn colorimeters.

Blood and Other Soft Tissues. The wet-digestion method suggested by Wilkins *et al.* (*11*) with slight modifications was preferred to the dry-ashing method because it is less time-consuming and a complete solution of the inorganic material is more easily effected.

Ten- to 20-gram samples are placed in 300-ml. Kjeldahl flasks and digested with 2 ml. of sulfuric acid with repeated additions of nitric acid until the major portion of the organic material is destroyed. The digestion is continued with 2 or 3 ml. of perchloric acid and additions of small quantities of nitric acid until the solution remains colorless. It was found that addition of the sodium chloride–hydrochloric acid mixture was not necessary. Boiling with about 50 ml. of metal-free water effects a complete solution of the digest. The specimen is transferred to a 125-ml. separatory funnel and the lead is extracted according to the procedure described for urine, except that the solution must stand for at least 2 hours with the sulfur dioxide, and preferably overnight, in order to insure the complete reduction of the ferric iron.

Bone. Specimens (2 to 3 grams) are digested with approximately 25 ml. of nitric acid and repeated additions of small quantities of hydrogen peroxide.

The digestion is completed by the addition of 5 ml. of perchloric acid and repeated additions of small quantities of hydrogen peroxide until the solution remains colorless. The lead is extracted from the bone digests in the same manner as from the

Table II. Recoveries of Known Amounts of Lead

(From 200 ml. of urine and 10 grams of blood with 50 micrograms of stannous tin and thallous thallium added to each specimen)

Lead Present[a]	Lead Added	Lead Recovered	Error
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
	Urine		
10	10	20.2	+0.2
10	10	20.8	+0.8
10	5	16.2	+1.2
10	5	15.7	+0.7
10	15	24.8	−0.2
10	15	25.9	+0.9
10	20	30.0	0.0
10	20	29.3	−0.7
10	25	35.6	+0.6
10	25	36.0	+1.0
10	17	27.5	+0.5
10	17	26.1	−0.9
	Blood		
2.3	2.5	5.1	+0.3
2.3	7.5	9.2	−0.6
2.3	12.5	14.5	−0.3
2.3	20.0	22.0	−0.3
2.3	25.0	26.8	−0.5
2.3	15.0	17.9	+0.6
2.3	5.0	8.2	+0.9

[a] Reagents contained 1.5 micrograms of lead. Lead present includes reagent blank and lead present in normal specimen.

Table III. Recoveries of Lead from 20 Grams of Blood with Varying Quantities of Added Bismuth

Lead Present[a]	Lead Added	Bismuth Added	Lead Recovered	Error
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
3.0	12.5	12.5	15.8	+0.3
3.0	10.0	10.0	13.6	+0.6
3.0	5.0	10.0	8.6	+0.6
3.0	2.5	15.0	7.0	+1.5
3.0	30.0	20.0	33.8	+0.8
3.0	12.5	15.0	15.2	−0.3
3.0	7.5	12.5	10.0	−0.5
3.0	5.0	25.0	8.8	+0.8

[a] Reagents contained 1.5 micrograms of lead. Lead present includes reagent blank and lead present in normal specimen.

digests of other tissues, except that it is necessary to add 20 ml. more of the sodium citrate to keep the large quantity of calcium in solution and to prevent precipitation of lead as lead phosphate.

Results

The recoveries of lead from urine and blood specimens in the presence of thallium and tin are given in Table II. The recoveries of lead in the presence of bismuth are given in Table III. It will be noted that lead can be separated quantitatively from bismuth with the above technique.

Summary

A simplified mixed-color dithizone method for the determination of lead in biological material is described. The treatment of the digest with sulfur dioxide serves a twofold purpose: It reduces the iron and hence prevents the decomposition of the dithizone, and it oxidizes the tin and hence inactivates one of the metals which combines with the dithizone under essentially the same conditions as lead. The method eliminates the necessity of removing the excess dithizone or preparing standard dithizone solutions. Consistent recoveries of ±1 microgram of lead are obtainable.

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Assistance in this research was furnished by the personnel of Works Projects Administration Official Project No. 65-1-53-2349 and also by a grant from the Wisconsin Alumni Research Foundation.

Analysis of a Single Drop of Liquid by Microfractionation

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IT IS frequently desirable and sometimes imperative to determine the composition of a drop of liquid. Fractionation is obviously desirable, but the method (*1*) now in use permits collection of but six to twelve fractions per drop, depending on the skill of the operator. Such limited information is of little value. This paper describes a procedure for collecting thirty to seventy fractions from a single drop. A graph of the boiling point of each fraction against the fraction number shows clearly the existence of one or more components and, with reasonable care, the percentage composition.

The utility of this method has been confirmed by using it as an ordinary experiment in an advanced course in laboratory technique. All graphs reproduced in this article are from the work of students who have first acquired experience on a benzene-xylene mixture of known composition and next shown their proficiency on a mixture whose components and proportions were unknown to them. Over half of the class exhibit a skill comparable to that illustrated by these graphs. An operator, whose performance with the mixture of known composition shows less proficiency, can still make creditable use of the method with unknown mixtures whose boiling points are further apart than those represented here, or with a drop in which one component is present in relatively large proportions. Save for constant-boiling mixtures the method will always reveal whether a drop consists of a single component or a number of compounds.

The basis for the development of this method is the procedure of Emich (*2*, *4*), whose technique has been accepted as standard, but numerous changes have been introduced. The fractionating capillary is tubular in order to provide a high ratio of surface to free space. It is supported in a vertical instead of an inclined position, and is packed with glass wool instead of being left empty. A copper block is substituted for a free flame as a source of heat. The column of the capillary is jacketed by a section of the block instead of being exposed to air.

Apparatus

Fractionating Capillaries. Two types of fractionating capillaries are illustrated in Figure 1. The first is a straight tube whose inside diameter is 1.5 to 2 mm. and over-all length is 13 cm. Soft-glass tubing (14 mm.) can be conveniently drawn to this size. The packing is glass wool, ground in a mortar until small enough to pass easily into the capillary tube, and inserted before the constriction is made. This constriction should be as short as possible, so that no volume of condensate larger than necessary will be trapped. A wet filter paper condenser is illustrated at the top.

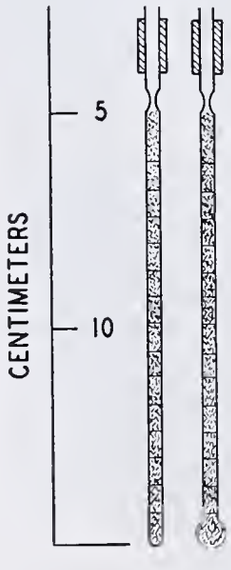


Figure 1. Fractionating Capillaries

The second type of fractionating capillary follows closely the conventional pattern of flask, column, and condenser used in macrowork. Construction is more difficult. Advantages are a lower liquid level in the capillary, a resulting increase in effective length of the column, and a larger distilling surface. Moreover, the bulb at the bottom facilitates removal of the insulating jacket.

Copper Heating Block. The copper block, pictured in Figure 2, *a*, is 3.8 cm. square and 15 cm. high. Two holes, 6 and 8 mm., respectively, are bored to a depth of 9.5 cm. The thermometer in the first hole is to facilitate control of operations, not to measure the boiling point. In reality, it will record a temperature higher than the boiling point of the lower boiling component. The fractionating capillary shown in the second hole is in practice surrounded by glass or asbestos tubing open at both

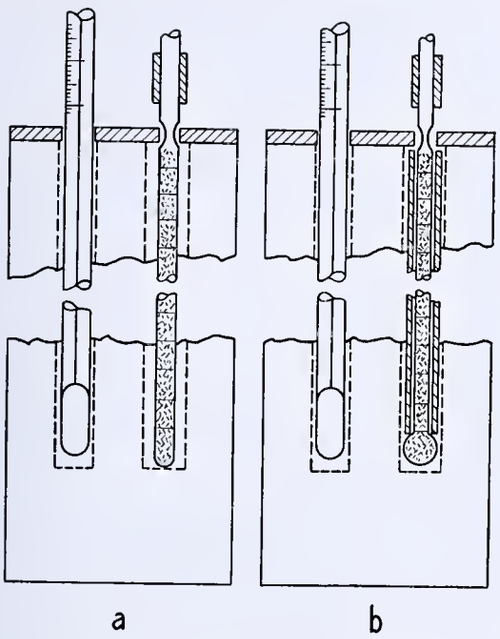


FIGURE 2. COPPER BLOCK USED FOR HEATING FRACTIONATING CAPILLARIES
Insulating jacket around capillary has been omitted from *a* in order to show better the various parts.

ends, which serves to insulate the capillary from the heated copper surface. With fractionating capillaries of the second type, the insulating jacket rests on top of the flask (Figure 2, *b*). A thin (approximately 1-mm.) sheet of asbestos covering the block reduces radiant heat which might otherwise prevent efficient cooling in the condenser. The asbestos fits snugly about the fractionating capillary and is cut from one edge to the center in order to facilitate its removal. A small gas flame directed against the bottom provides satisfactory heating. Electrical heat, produced from 12.2 meters (40 feet) of No. 30 oxidized resistance wire wrapped around the lower 4 cm. of the block and regulated by a variable transformer, is better.

BOILING-POINT CAPILLARIES. The form described by Emich (*2*) has in general been used. He prescribed a bore of 0.5 mm. or more. The authors experience no difficulty with tubes whose diameter is as small as 0.2 mm.; indeed, the smaller the bore the greater the number of fractions which can be collected from a single drop. The smallest permissible bore will vary with the patience and skill of each operator. A fragile boiling point capillary, with the even more fragile tip, which one man can handle safely is broken by another. The corresponding tiny droplet, seen readily by one, cannot be observed so easily by another. Much trouble will be avoided if each experimenter will first construct tubes of varying size and determine with a sample of pure benzene or other liquid the smallest size with which he can obtain consistent results.

The tip should be not less than 1 cm. long. The 2-cm. length prescribed by Emich is satisfactory but harder to make with so small a tube. The longer the fine tip, the less the liquid lost by evaporation during sealing. Soft-glass tubing, 10 mm. in diameter, is suitable for drawing the capillaries. The fine tip at the end is made with the aid of a microflame from a thin-walled capillary tubing whose diameter is about 0.8 mm.

Forty or more of these boiling point capillaries, having as nearly uniform size as possible, are constructed. They can be kept in order by inserting them in small numbered holes bored in a large cork or punched through the two halves of a sheet of notebook paper folded at the middle and standing on the two long edges.

Determination of Boiling Points

The boiling points for Figures 3, 4, and 5 were determined in a liquid bath similar to that described by Emich (*2*) and in greater detail by Gettler (*3*) and co-workers. All other determinations were made in a copper-block apparatus (*5*).

Fractionation

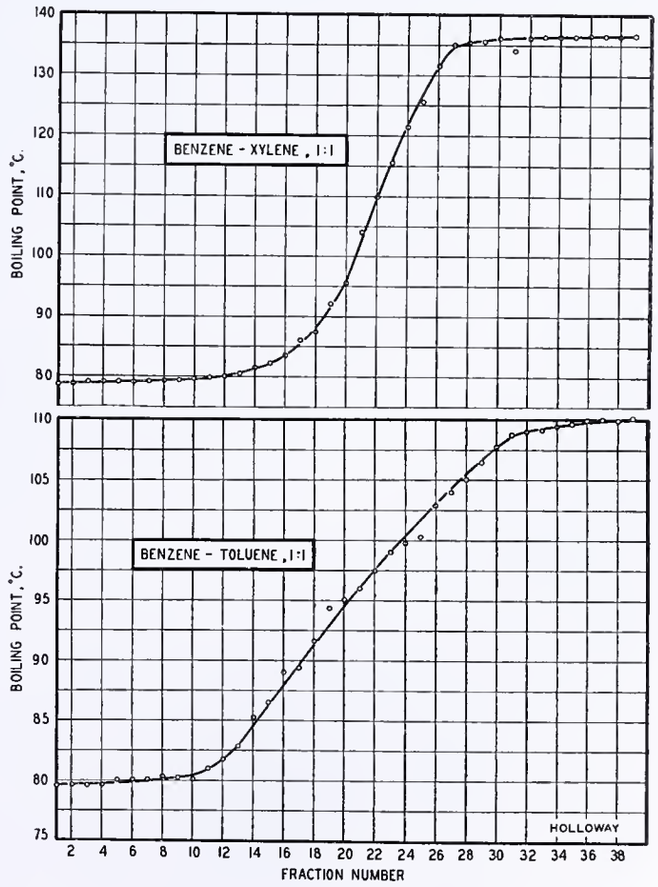
An operator should not attempt fractionation of an unknown mixture until he has first demonstrated his ability to distill and collect satisfactory samples from the fractionating capillary, using a drop with a single component such as benzene; and secondly, perfected his skill by practicing on a drop of known composition. These two preliminary trials should employ the technique to be used for the unknown sample.

The fractionating capillary is first weighed on an ordinary analytical balance. A single drop of liquid is pipetted into the tube, which is then centrifuged until the drop is forced through the constriction to the bottom of the capillary. The tube is weighed again in order to determine the weight of the sample. The paper condenser is next attached, the insulating jacket put in place, and the fractionating capillary inserted in the block.

Heat is applied gradually. The filter paper is wet and a fine stream of air directed against it to provide further cooling. When the first tiny droplet appears above the constriction, heating is discontinued, the asbestos cover pulled aside, and the fractionating capillary removed and centrifuged. An electrically operated centrifuge is preferred because the operator can continue to centrifuge while using this period to shut off the heat and cool the insulating jacket. Cooling is effected by a stream of air or by removal of the insulating jacket. In the latter case, the advantage of a fractionating capillary of the second type is apparent, for the glass or asbestos tube comes out with the capillary. After centrifuging, the capillary is replaced inside the jacket in the block. These operations need not be hurried. Three or four minutes of centrifuging may elapse before the fractionating capillary is replaced. The temperature of the block should also have fallen 4° to 5° during this period.

The whole sequence of operations, consisting of heating until a droplet is condensed, removing capillary, shutting off heat, centrifuging, cooling the jacket, and heating again is repeated three or four times. In each case care is taken to find the lowest temperature at which enough liquid for a determination will condense in the constriction within 1 or 1.5 minutes.

A fraction is next collected by touching the tip of the capillary boiling tube to the droplet. Heating is then reduced or discontinued and the fractionating capillary removed and put in the centrifuge. The small tip of the capillary boiling point tube is sealed. The boiling point can now be determined at once.



FIGURES 3 (*Above*) AND 4 (*Below*). BOILING POINTS OF BENZENE-XYLENE AND BENZENE-TOLUENE MIXTURES

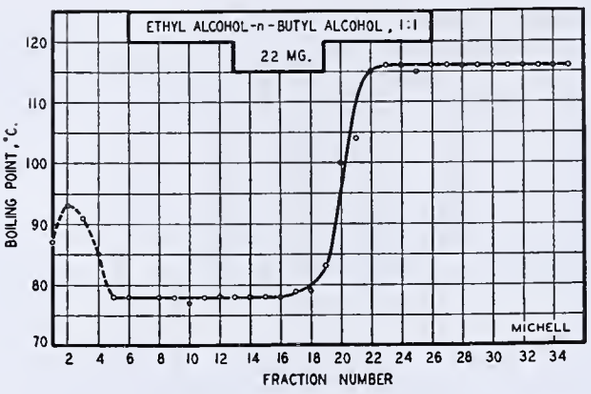


FIGURE 5. FRACTIONATION OF ETHYL ALCOHOL–BUTYL ALCOHOL DROP

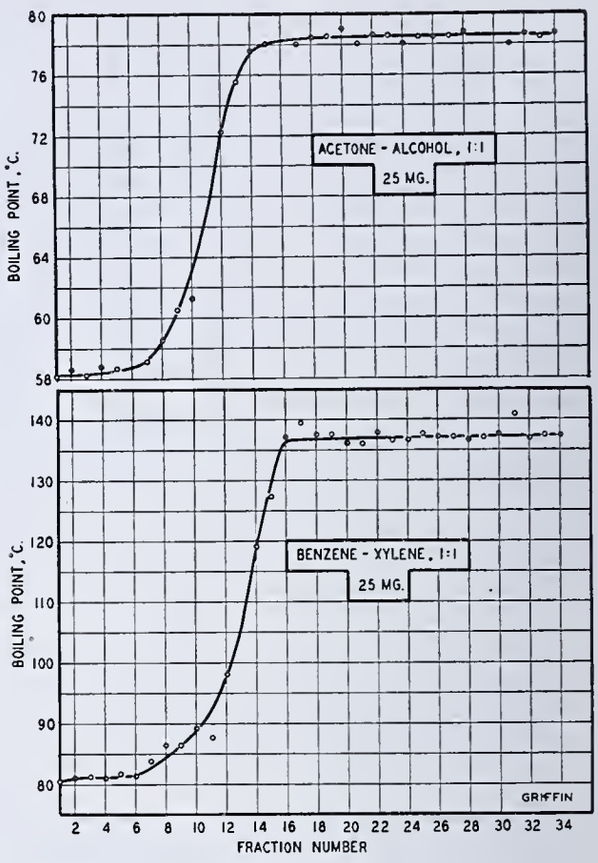
While making the necessary observations the insulating jacket is cooled. After observing the boiling point the fractionating capillary is taken from the centrifuge and replaced in the insulated jacket of the copper heating block preliminary to distilling the next sample. The block should not have cooled more than 2°. With some care all operations can be synchronized (not necessarily in the above order), so that the boiling point of one droplet can be determined before collection of the next. Such perfection of operations is ensured by having all apparatus assembled so compactly that minimum movement is required and by never permitting the temperature of the bath (used in determining boiling points) to fall more than 2° C. below the boiling temperature of the sample, so that little time is lost in this determination. After collecting the first two or three droplets, the process should be in smooth operation.

The number of fractions obtained varies with the individual. As many as seventy droplets[1] have been collected from a single drop weighing approximately 25 mg.; the usual number is thirty to thirty-five. The time required to fractionate and to observe all boiling points is 4 or 5 hours. Here also the time needed to perform rapid yet satisfactory work varies. When proper precautions are taken, however, a reasonably good separation is always possible.

A major trouble arising from loss of condensate, particularly with the low-boiling portions, can usually be traced to failure to keep the condenser cold; failure to reduce radiant heat from the top of the block; or evaporation losses from too long a period for distilling and collecting a sample. If evaporation of water fails to cool the paper condenser properly, ether may be used. Suction, instead of an air jet, should then be employed in order to keep the laboratory free from ether vapor. Routine mechanical attendance to the condenser is reduced by feeding the liquid to the paper condenser through an extremely fine capillary tip from a dropping funnel. An effective condenser has also been constructed from No. 13 copper wire, one end of which is wrapped about the glass condenser surface and the other end is wrapped in the shape of a basket or cone in order to hold a few lumps of solid carbon dioxide. The relatively heavy copper wire causes the condensing surface to be maintained at a very low temperature.

Another error may be caused by an overheated jacket and is revealed by the general absence of fractionation, even though the normal number of droplets are collected. The source of heat for distillation should be at the bottom of the tube. Too high a temperature in the jacket means that distillation occurs from the middle instead of from the bottom of the column. The temperature of the block will be 10° C. or more higher than the boiling point of the fraction being collected. The method of cooling the jacket between collections of samples is a compromise with a more time-consuming

[1] Since completion of the manuscript one of the students, Mr. Towle, has succeeded in obtaining 106 fractions from a drop weighing 23 mg.



FIGURES 6 (*Above*) AND 7 (*Below*). BOILING POINTS OF ACETONE-ALCOHOL AND BENZENE-XYLENE MIXTURES

operation and complicated apparatus required if the jacket temperature were accurately controlled. If the time for collecting a fraction is prolonged much beyond the minute or minute and a half specified, the jacket becomes too hot.

Lack of patience and care contributes considerably to poor results. Leisurely but steady work is better than a hurried pace.

Some additional refinements are possible. When it is apparent that the temperature is rising to a higher boiling fraction, greater care can be exercised to get minimum temperature for distillation, even if it is necessary to remove the tube, centrifuge, etc., a second time before collecting the next sample. It is also possible to keep better account of the amount collected by weighing each boiling point tube as the droplet is removed. Such operation becomes very tedious. With skill in making boiling point capillaries of uniform size and some practice in manipulation, it is possible to collect droplets of reasonably uniform volume. Boiling points of

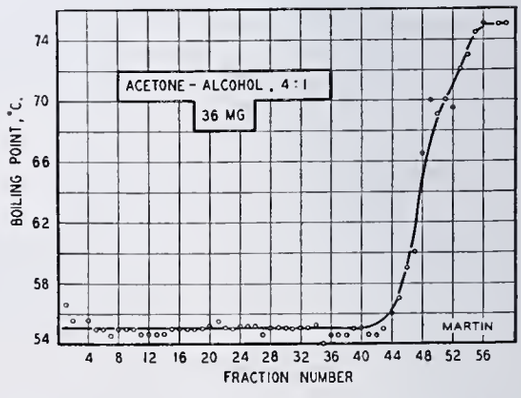


FIGURE 8. FRACTIONATION OF ACETONE-ALCOHOL MIXTURE

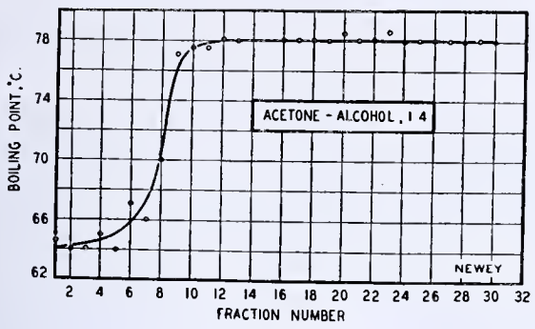


FIGURE 9. FRACTIONATION OF ACETONE-ALCOHOL MIXTURE

each fraction may then be plotted against the fraction number.

Greater accuracy can also be had by taking a larger sample with corresponding lengthening in time. When the quantity of one component falls to 20 per cent or lower, a larger sample (two drops, for example) is necessary if the boiling point of the smaller component is desired. Similarly, two drops would be necessary if a three-component mixture were being fractionated.

Results

Figures 3 to 12, inclusive, exhibit typical results of first-class work. Fractionation of unknowns in all instances was preceded by tests upon a known mixture of equal parts of benzene and xylene. Three of these preliminary trials are included (Figures 3, 7, and 9) because they show, by comparison with the adjacent graph obtained by the same experimenter for the unknown, the consistency with which an operator can reproduce his results.

Figures 3 and 4 form such a pair, the first on the trial mixture, the second on the more difficult benzene-toluene combination. The boiling points in each instance were observed in the conventional liquid bath. Assuming that fraction 22, half-way between the boiling point levels, in Figure 3 represents the mid-point, the composition would be 56 per cent benzene. If fraction 20 is the mid-point of the benzene-toluene pair, the composition would be 51 per cent benzene. No correction is made in either case for holdup in the column.

Figure 5 pictures the fractionation as an unknown of a drop containing equal parts of ethyl alcohol and *n*-butyl alcohol. The irregularity of the first four points is caused either by failure to heat, centrifuge, and reheat the microcolumn often enough to ensure constancy of operating conditions or by the slower rate at which the operations are necessarily performed during the first period. A similar effect on a smaller scale can be seen in Figure 9. Estimated composition on basis of a mid-point at the twentieth drop is 57 per cent ethyl alcohol with no correction for an assumed holdup.

Figures 6 and 7 show another pair of practice and unknown tests. The fractionations are particularly good as far as correctness of boiling point and sharpness of cuts are concerned. The percentage compositions are consistent with each other, although incorrect in absolute values. The difficulty is due to one of three possible causes: loss of some of the lower boiling component by ineffective condensing; collection of larger fractions at the beginning than were collected at the end of fractionation; or a personal factor on the part of the operator.

Figures 8 and 9 show extremes on two acetone-alcohol mixtures with different compositions. In both cases it proved impossible to get a correct value for the boiling point of the component whose concentration was as low as 20 per cent. The percentage compositions, however, were estimated with considerable accuracy, being 81 per cent acetone with the first mixture and 73 per cent alcohol with the second. No correction for holdup was applied in either case.

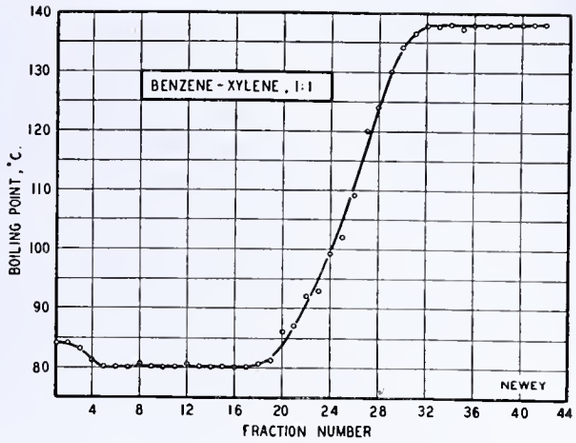


FIGURE 10. FRACTIONATION OF BENZENE-XYLENE MIXTURE

Figure 10 portrays the trial mixture which accompanies the results in Figure 9. Using the data in this practice test to make a correction for holdup in Figure 9, the results would be 80 per cent alcohol content, in agreement with the proportion used in the mixture. Such accuracy is not claimed in behalf of this process. The value of 73 per cent (uncorrected) on a mixture having 80 per cent of one component is, in fact, good work when the many variables in manipulation of this type are considered. Figure 10 also shows by comparison with Figure 3 the satisfactory results obtained in making determinations of boiling points in a copper block (*5*) instead of in a liquid bath.

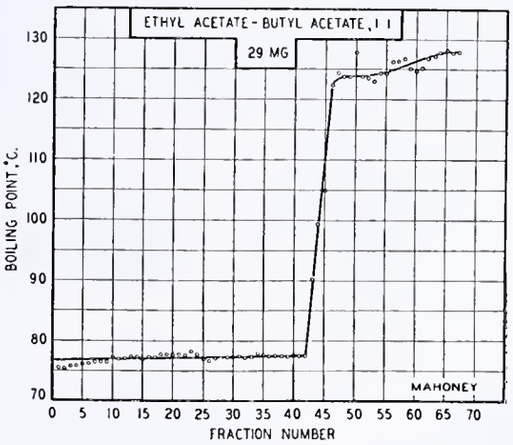


FIGURE 11. FRACTIONATION OF ETHYL ACETATE-BUTYL ACETATE MIXTURE

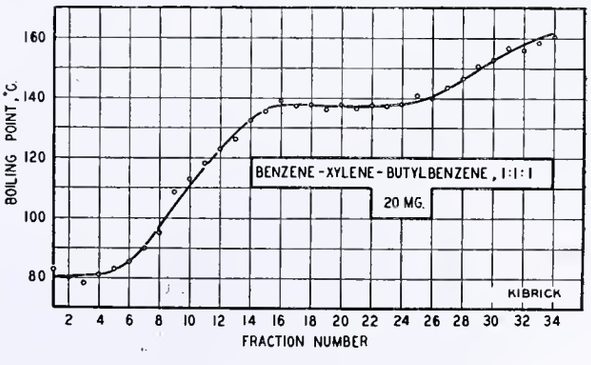


FIGURE 12. FRACTIONATION OF THREE-COMPONENT MIXTURE

Figure 11 illustrates the results of fractionating, as an unknown, a drop of a mixture of equal volumes of ethyl acetate and butyl acetate. The work shows the very large number of fractions that can be collected from a single drop. Each fraction weighed not more than 0.43 mg. This determination was stopped as soon as a constant boiling point of the butyl acetate portion was assured.

In Figure 12, the boiling points of two of the three components present are judged correctly. Presence of at least three substances is clearly evident. The results show that a satisfactory determination can be made on a component present to the extent of 33 per cent only. The minimum concentration for which correct determinations can be made by this method lies between the 20 per cent level observed in Figures 8 and 9 and the 33 per cent content in Figure 12.

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CONTRIBUTION No. 244 from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology.

Copper Blocks and Optical System

For Determining Boiling Points (Emich Method) and Melting Points

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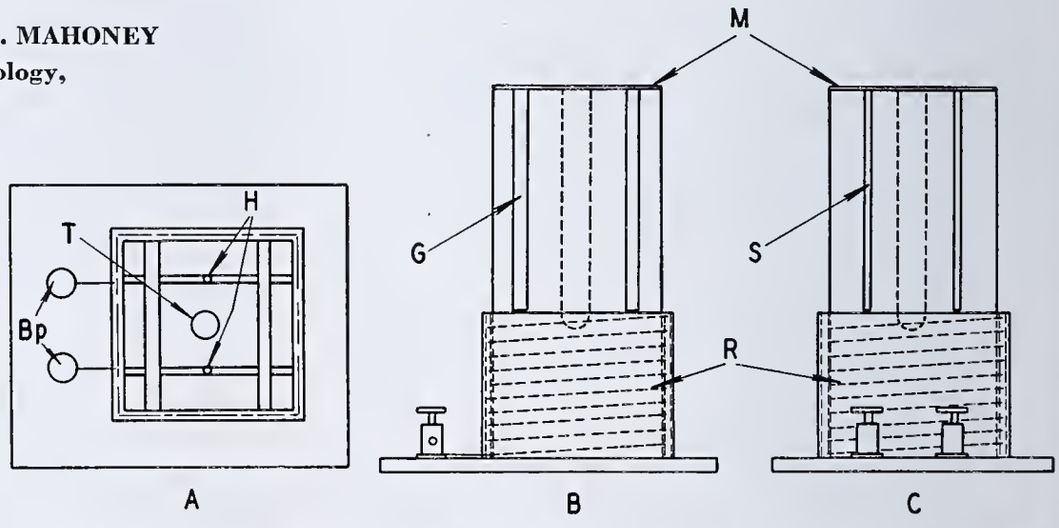

FIGURE 1. CONSTRUCTION OF COPPER BLOCK FOR USE WITH BOILING POINT CAPILLARIES

A, top view. *B*, side view parallel to light beam. *C*, side view at right angles to (facing) light beam. Visual observation can be made through slits *S* if a strong light is placed on the far side. *T*, thermometer hole. *H*, holes for boiling point capillaries in mica cover plate. *M*, mica cover plate. *G*, Pyrex glass plates 3.5 mm. thick for passage of light through block. *R*, resistance wire (40 feet of No. 30). *Bp*, binding posts. Block is 3.8 cm. across and 10.2 cm. high.

OBSERVATIONS of boiling points in capillary tubes by the Emich method (*1*) involve use of a liquid heating bath. The possibilities in cleanliness and high temperatures make the construction of a metal block very desirable. Figure 1 shows a form which has proved very useful. By means of an optical system (Figure 2) the inverted image of the boiling point capillary and drop can be observed on a paper screen with a minimum of eyestrain.

The determination of the boiling point in this apparatus need not vary from the usual practice in a liquid bath. The block is heated by the resistance wire until the droplet appears above the surface of the metal. Heating is then discontinued, the block is cooled by the air jet, and the determination is repeated until the values can be duplicated.

When the apparatus is used for a series of boiling points as in the fractionation (*2*) of a drop of liquid, the repeated observation on a single fraction consumes too much time in a process which is itself time-consuming. In such a case the block is heated to within about 5° of the boiling point before insertion of the boiling point capillary. A small amount of vapor sometimes condenses in the capillary above the surface and hinders the upward movement of the droplet through the length of the tube. This difficulty can often be avoided by breaking the capillary until it protrudes just above the surface, so that the vapor escapes. Such treatment occasionally results in a failure to get any value for one or two of

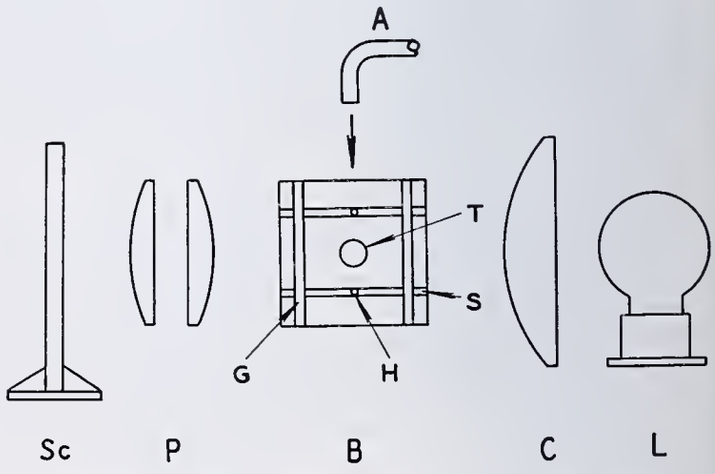

FIGURE 2. OPTICAL SYSTEM FOR BOILING POINT DETERMINATION

Sc, screen. *P*, projection lens. *B*, top view of copper block for boiling point determination. *A*, air blast directed against side of block. *T*, *S*, *H*, and *G*, as in Figure 1. *C*, condenser lens, 8.5 inches focal length. *L*, 100 watt lamp.

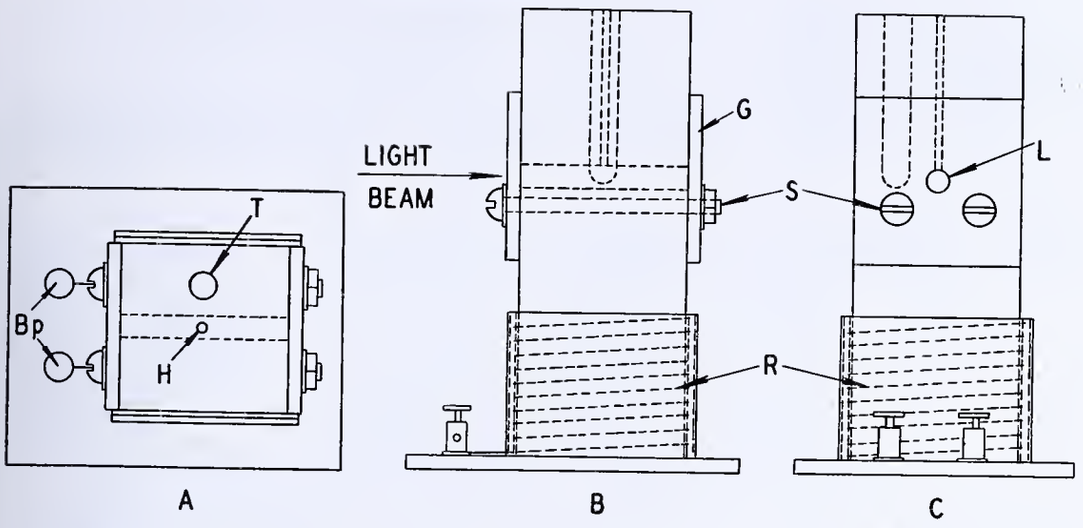

FIGURE 3. MELTING POINT BLOCK FOR USE WITH OPTICAL SYSTEM

A, top view looking down upon mica cover glass showing thermometer with cross slit and holes for capillary melting point tube. *B*, side view facing beam of light. *C*, side view parallel to light beam. *H*, 1.5-mm. hole for melting point tube. *L*, 5-mm. hole for light. *G*, Pyrex glass plates fastened to copper block by cross bolts, *S*. *R*, resistance wire (40 feet of No. 30). *Bp*, binding posts. Blocks are 3.8 cm. across and 10.2 cm. high.

the fractions, but the loss is not serious where so many determinations are made and a curve is plotted. Each separate determination in a series can usually be made more accurately than is possible in an isolated case, for the operator soon acquires a perfection in every detail of operation that is difficult to attain in individual cases.

This apparatus was used in most of the observations recorded by the authors (*2*).

MELTING POINT BLOCK. The authors constructed a copper block from 1.5 inches (38 mm. square) of copper for use with the same optical system (Figure 3). The relatively large opening in the side needed for the use of the optical system is protected by two Pyrex glass plates fastened closely to the side of the block by bolts as illustrated.

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CONTRIBUTION from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology, No. 245.

A Photometric Method for the Determination of Magnesium

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IN A recent investigation involving the analysis of several soil extracts and fertilizers, it was necessary to determine fairly small quantities of magnesium. As a rapid and reliable method was desired, it was concluded that a colorimetric method, if sufficiently sensitive, might afford the most suitable procedure.

Several organic compounds which give color reactions with magnesium were studied, but none yielded as sensitive a test for this ion as did titan yellow. In an alkaline solution magnesium ions react with this compound to produce a red or pink color, which can be readily matched with standards or read in a photoelectric colorimeter. The use of titan yellow for the determination of magnesium was first suggested by Kolthoff (*2*), who stated that it was capable of detecting 0.2 part of magnesium per million of solution. He suggested using it in the analysis of tap water but reported only one determination.

In this investigation the method has been adapted to soil and fertilizer analysis and to a photoelectric colorimeter.

[1] Present address, Purdue University, Lafayette, Ind.

Preliminary Investigation

Some difficulty was originally encountered in retaining the pink color developed after the addition of sodium hydroxide. In many instances the color faded rapidly and consistent readings could not be obtained; a small amount of hydroxylamine hydrochloride effectively stopped the fading.

In order to determine the stability of the color developed, various solutions of known concentration were read at intervals in the photometer over a period of several hours. The instrument was set on zero per cent light absorption and if necessary, adjustments were made to bring the galvanometer to its null point after each reading. The results obtained were very consistent—for example, one solution containing 1 p. p. m. of magnesium read 31, and when read at 20-minute intervals for the next 3 hours gave 31.0, 31.0, 31.0, 30.8, 30.8, 30.8, 30.5, and 30.5.

Solutions containing 4 p. p. m. of magnesium were found to be stable for 1.5 hours, whereas solutions containing 3 p. p. m. of magnesium were stable for 4 hours, and the more dilute solutions were stable for over 12 hours.

Ammonium ions up to a maximum concentration of 500 to

600 p. p. m. did not disturb the reaction, but concentrations of aluminum and tin as low as 4 p. p. m. partially destroyed the color and resulted in low readings. Phosphate ions were found effective in destroying the color, if their concentration was in excess of 100 p. p. m.

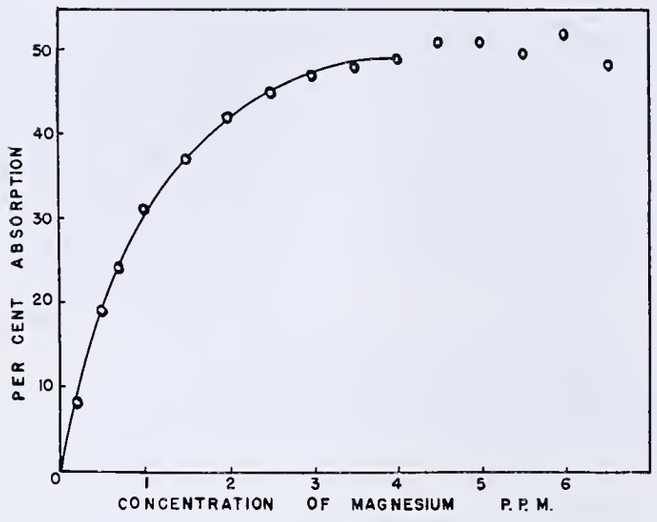

FIGURE 1. CALIBRATION CURVE FOR MAGNESIUM SULFATE

The curve in Figure 1 was originally calibrated in the presence of sucrose. It was believed this would aid in minimizing the effect of the calcium ion, and it was determined that calcium, in the presence of sucrose, up to a maximum concentration of 800 p. p. m. did not affect the readings—that is, 1 p. p. m. of magnesium could be determined in the presence of 800 p. p. m. of calcium. However, to obtain the greatest accuracy it is advisable to keep the concentration of calcium below 500 p. p. m. With no sucrose present calcium did not interfere until the concentration reached 500 p. p. m.

For accurate analysis 3 p. p. m. of magnesium is the maximum concentration that can be present in the solution being read in the colorimeter.

The light filter used was a Wratten No. 58, and its spectral absorption curve was determined by means of a spectrophotometer (curve 1, Figure 2). Since maximum transmission of light occurs at 530 millimicrons and maximum absorption of light by the colored complex occurs in approximately the same region (curve 2, Figure 2) it is apparent that the filter used was suitable for this particular color complex.

Method

REAGENTS. Titan yellow solution consisting of 0.15 gram of the dye dissolved in 75 cc. of 95 per cent ethanol and 25 cc. of distilled water.

Standard solution of magnesium sulfate, containing 100 p. p. m. of magnesium.

Four per cent solution of hydroxylamine hydrochloride, 5 per cent solution of sucrose, and 4 per cent solution of sodium hydroxide.

PROCEDURE. Iron, aluminum, and phosphorus are removed by precipitation as iron and aluminum phosphates (*4*). Calcium is removed by precipitation as the oxalate.

The filtrate from the above separations is evaporated to dryness, the ammonium salts are destroyed by ignition, and the residue is taken up in water. If the residue dissolves with difficulty a few drops of acid are added.

The solution is then made to volume and an aliquot is pipetted into a 100-cc. volumetric flask. Exactly 10 cc. of a 5 per cent sucrose solution and 2 cc. of a 4 per cent solution of hydroxylamine hydrochloride are added by means of pipets. Ten drops of the titan yellow solution are added and the contents of the flask are diluted to approximately 70 cc. with distilled water. Finally, 10 cc. of a 4 per cent solution of sodium hydroxide are pipetted into the flask while the contents are gently agitated. The color develops immediately and the solution is then made to volume, mixed thoroughly, placed in the optical cell, and read in the photometer. The concentration of magnesium is obtained from the calibration curve, Figure 1.

The instrument can be set on zero per cent light absorption and the galvanometer brought to its null point against either distilled water or a solution containing 10 drops of titan yellow and 10 cc. of a 4 per cent solution of sodium hydroxide. The latter solution was used in this investigation.

STANDARD CURVE. Figure 1 shows the standard curve for magnesium sulfate solutions obtained with a Fisher photometer using scale No. 1, and a Wratten green light filter No. 58.

Aliquots of the standard solution were taken containing 0.2, 0.5, 0.7, 1.0, 1.5, etc., p. p. m. of magnesium and were analyzed exactly as described above. To illustrate the reproducibility of the results, the data obtained during the calibration trials are given in Table I. The average figures in the right-hand column were used in plotting the curve in Figure 1.

TABLE I. PHOTOMETER READINGS CORRESPONDING TO DIFFERENT CONCENTRATIONS OF MAGNESIUM

Magnesium Concentration	Photometer Readings								
	1	2	3	4	5	6	7	8	Av.
P. p. m.									
0.2	8	9	8	7	7	7	..	..	8
0.5	19	18	19	19	19	18	18	..	19
0.7	24	24	25	24	23	24	..	..	24
1.0	30	31	31	31	31	31	32	31	31
1.5	37	37	37	37	..	..	..	..	37
2.0	42	41	41	43	43	42	40	42	42
2.5	45	45	45	45	45	..	..	..	45
3.0	46	46	47	47	47	..	..	..	47
3.5	49	49	49	47	46	48	..	..	48
4.0	49	49	48	49	48	48	47	..	49

Analytical Results

The method was used in the analysis of several commercial fertilizers, water, and six soil extracts, and the results are listed in Tables II and III. The magnesium was extracted from the 2-12-6 fertilizers by means of concentrated acids, and the phosphorus, aluminum, and calcium were removed as directed above.

Very excellent agreement between duplicates was obtained (Table II). With three samples results were identical, whereas with samples 1, 4, and 6 a deviation of 2.2, 3.8, and 6.6 per cent, respectively, was noted. With four of the samples different sized aliquots were taken and these results were almost identical with the other determinations. The analyses are therefore independent of the size of the aliquot.

Similarly, very excellent agreement among the triplicates run on tap water was obtained. This method is especially suited to water analysis, since it is very sensitive and rapid, and interfering ions are not ordinarily present in sufficient quantities to necessitate their removal.

TABLE II. DETERMINATION OF MAGNESIUM

Fertilizer No.	Magnesium Found		
	1	2	3
	P. p. m.	*P. p. m.*	*P. p. m.*
	Oven-Dry 2-12-6 Fertilizers		
1	525	533	538[a]
2	2000	2000	...
3	125	125[a]	...
4	313	325[a]	320
5	875	875[a]	...
6	75	80	80
Sample	Water		
1	12.50	12.50	12.75
2	5.75	5.70	5.70
3	3.00	3.00	3.00

[a] Different sized aliquots were used in these samples.

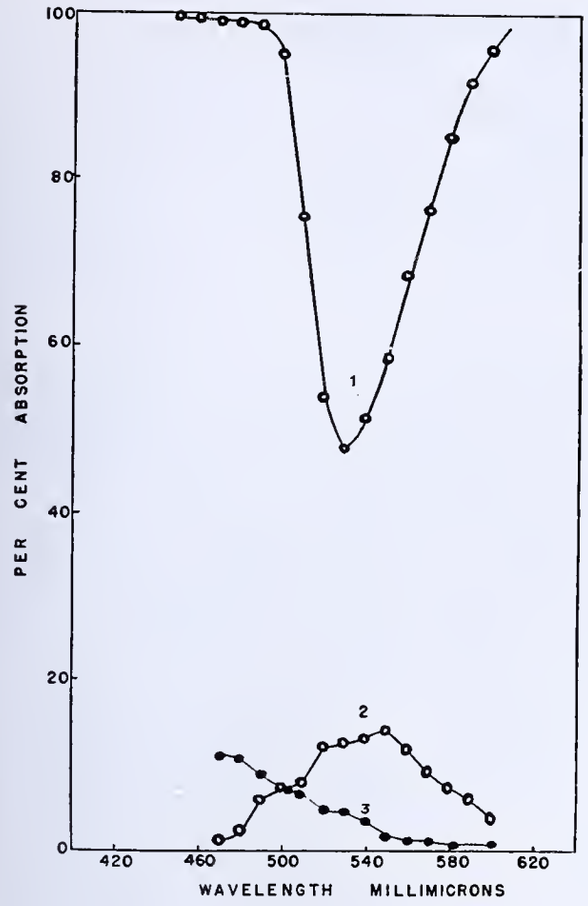

FIGURE 2. SPECTRAL ABSORPTION CURVES

1. Wratten green light filter 58
2. Color complex
3. Titan yellow

Exchangeable magnesium was determined on several soil samples by both gravimetric and colorimetric methods. The magnesium was displaced by leaching the soil with a neutral solution of normal ammonium acetate, the leachates were made to volume, and a 50-cc. aliquot was taken for the colorimetric analysis. Magnesium was determined gravimetrically on the remaining solution by precipitation as magnesium hydroxyquinolate (*3*). The results (Table III) show good agreement between the two methods. The maximum difference, about 8.5 per cent, occurs in sample 6, whereas the error for the other samples runs around 1 per cent or less.

TABLE III. DETERMINATION OF EXCHANGEABLE MAGNESIUM IN SOIL EXTRACTS

Sample	Colorimetric 1	Colorimetric 2	Colorimetric 3	Gravimetric[a]
1	7.00	7.00	7.00	7.03
2	7.00	7.00	6.50	6.90
3	5.50	5.50	5.80	...
4	11.60	11.40	11.20	11.53
5	10.00	9.50	9.80	9.68
6	8.50	8.20	8.50	7.68

[a] Acknowledgment is due Kirk Lawton, who determined the magnesium gravimetrically as magnesium hydroxyquinolate.

Discussion

The curve in Figure 1 flattens out in the neighborhood of 4 p. p. m. and for this reason the upper limit of concentration is set at 3.0 p. p. m. of magnesium. This flattening of the curve might appear as a possible weakness in the method, because on the level portion one could not judge whether he was dealing with concentrations of magnesium in the neighborhood of 4 p. p. m. or greater than 6.5 p. p. m. However, the point at which the curve levels off is easily detected, since a red, flocculent color lake appears almost immediately if the concentration of magnesium exceeds 4 p. p. m. Thus, if a solution gave a photometer reading of 48 or 49 a precipitate would appear with solutions containing around 6.5 p. p. m. Consequently, the aliquot ean be quickly adjusted to the proper concentration.

The solubility of magnesium hydroxide is approximately 9 mg. per liter, which corresponds roughly to 4 p. p. m. of magnesium. At pH 13, however, the theoretical solubility of magnesium hydroxide (from solubility product calculations) is about 10^{-5} milligram per liter. The solubility may be larger under the experimental conditions because of the buffering action of hydroxylamine, or the possible formation of a complex with the titan yellow. The ultramicroscope showed the presence of colloidal particles in all the solutions examined.

Titan yellow is usually considered as an adsorption indicator in this reaction. Ginsberg (*1*) found that a state of equilibrium existed between the dyestuff and the so-called color lake, and from his equilibrium data concluded that a definite lake was not formed, since the lake formation follows the simple laws of adsorption.

As the maximum concentration of magnesium that can be present in any aliquot is 3 p. p. m., this method might appear applicable only over a very narrow range in concentration—namely, 0.5 to 3.0 p. p. m. of magnesium. However, as the size of the aliquot may be varied from 75 to 1 cc., the concentration range of the method actually lies between 0.5 and 300 p. p. m. of magnesium.

The procedure involved in this method is simple, and many samples can be analyzed in a very short time. It is much more sensitive and rapid than any gravimetric or colorimetric method in common use. It has given consistent results in this laboratory, but other materials should be investigated and further comparisons with other methods should be made.

Summary

A colorimetric method, based on the use of titan yellow and a photoelectric colorimeter, affords a rapid and reliable means for determining magnesium in quantities ranging from 0.5 to 300 p. p. m. in fertilizers and soil extracts. The colored complex is stabilized by hydroxylamine hydrochloride.

The procedure does not involve the separation and purification of any magnesium precipitate and thus eliminates several steps.

Calcium, up to a maximum concentration of 800 p. p. m., does not interfere with the determination of 1 p. p. m. of magnesium, but should be kept below 500 p. p. m. Similarly, ammonium and phosphate ions should be kept below 600 to 700 and 100 p. p. m., respectively, while aluminum and tin must be absent.

The spectral absorption curves for the colored complex and titan yellow were determined by means of a spectrophotometer. In the analysis of several commercial fertilizers, tap water, and soil extracts good agreement between duplicate determinations was obtained.

Results were found to agree well with those obtained by the hydroxyquinolate gravimetric method.

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CONTRIBUTION from the Soils Section, Michigan Agricultural Experiment Station. Authorized by the Director for publication as journal article No. 467 n. s.

Micromethod for Identification of Volatile Liquids

Vapor Pressures of Cyclopentane and the Pentenes

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IN THE course of a research on the photochemical decomposition of cyclic ketones, it became necessary to separate and identify very small quantities of volatile hydrocarbons. In this paper is described an apparatus for measuring the molecular weight, vapor pressure over a range of temperatures, and density of a liquid. As little as 5 mg. can be used and identified from the above properties. This method, together with the microanalysis technique of Blacet *et al.* (*1–5*) for gases, now constitutes a complete system for the identification of small amounts of products in kinetic reactions.

Apparatus

The unknown mixture of products was first fractionated by pumping it through a series of traps cooled to the appropriate temperatures. Where the mixture had a negligible vapor pressure in liquid air, the pumping was accomplished by using liquid air on the last trap. Where it contained permanent gases such as carbon monoxide or hydrogen, these gases were removed by pumping the sample through liquid air with a Toepler pump. Once they were removed, the separation could be carried on as previously described. In this manner a first fraction was obtained containing C_1 gases, such as carbon monoxide and methane together with any hydrogen. By the use of a Dewar flask containing freezing ethyl alcohol on the trap, the C_2 and C_3 fractions were isolated, and a similar procedure was used for the remaining higher hydrocarbons.

By this method of separation the higher boiling fractions were contaminated by at most 3 mole-per cent of the nearest lower boiling fraction. This impurity could be removed by a further isothermal three-step, bulb-to-bulb distillation. For fractions containing liquids boiling close to each other, separation was achieved by isothermal distillation from a 40-cm. vacuum-jacketed column of 3-mm. bore.

Once separated, the fractions were distilled individually into trap *T* (Figure 1). By now immersing this trap in baths at various temperatures, the vapor pressure of the fraction could be measured over a range of temperatures (static method). The sample was then expanded to fill the system at room temperature and the pressure was measured. Knowing the volume of the system, the volume of the sample at normal temperature and pressure could be calculated, using the ideal gas law. By adjusting the volume of the system, the gas could be made sufficiently dilute so as to be ideal. For the C_5 hydrocarbons, the pressures were below 30 mm. of mercury.

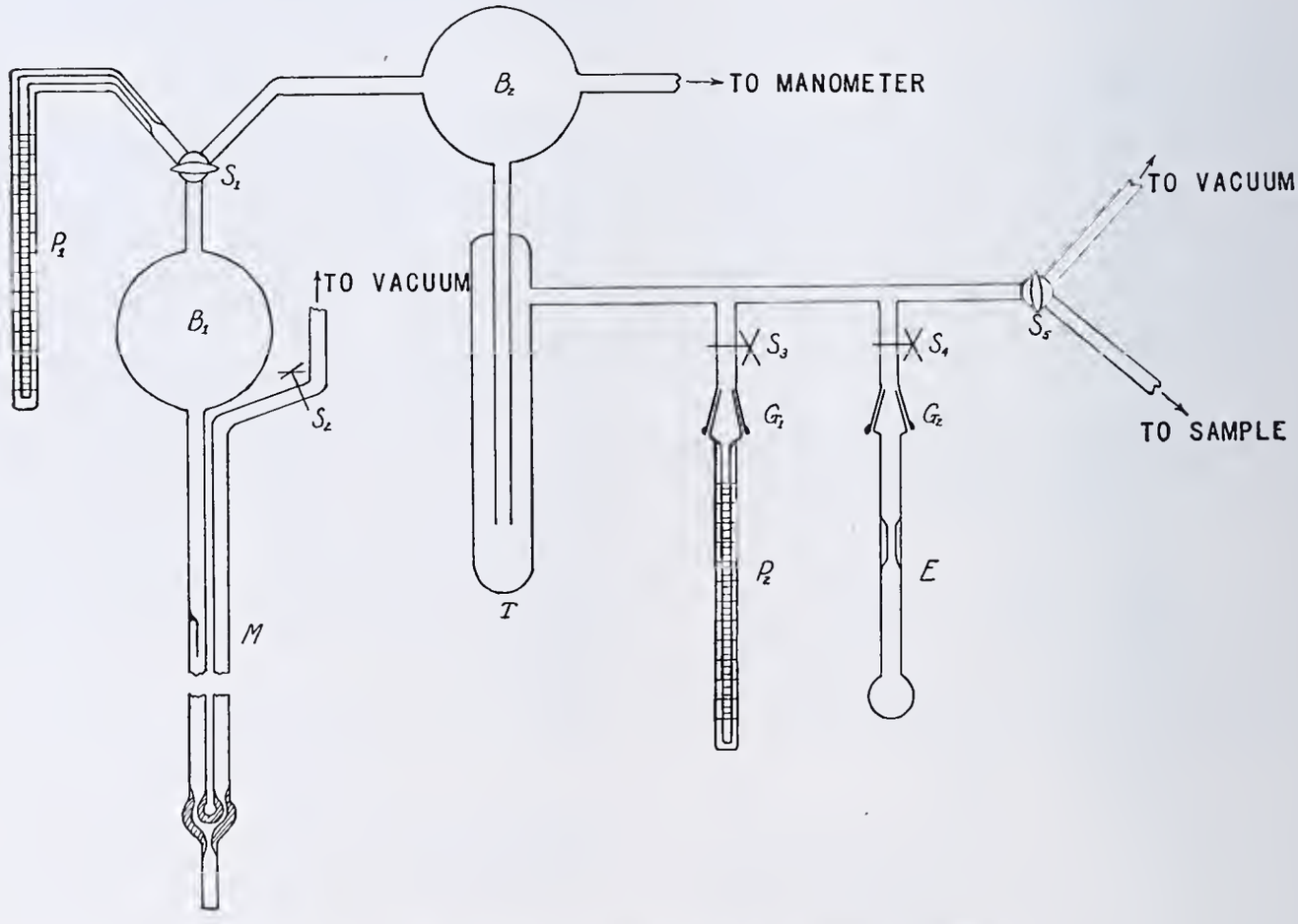

FIGURE 1. APPARATUS FOR MEASUREMENT OF PHYSICAL CONSTANTS

B.	100-cc. bulbs	*M.*	Manometer
B_1.	Toepler pump	*P.*	Micropycnometers
E.	Weighed bulb	*S.*	Stopcocks
G.	Ground-glass joints	*T.*	Trap

The sample was then frozen out in the micropycnometer, P_2, with liquid air. This micropycnometer is a capillary tube, sealed at one end and connected to the apparatus by a ground-glass joint. It is graduated in millimeters and calibrated by weighing drops of mercury. A few were constructed, with diameters varying from 0.75 to 3 mm. to hold larger or smaller quantities of liquids. By immersing it in baths at various temperatures the liquid volume of the fraction can be measured together with the coefficient of expansion. The stopcock above the pycnometer, S_4, is shut to prevent the liquid from distilling into the rest of the system. From the vapor pressure of the fraction, already obtained, and the volume of the micropycnometer one can correct the liquid volumes for the vapor above the liquid. At the higher temperatures this correction amounts to a few per cent.

The liquid is now distilled into the weighed bulb and sealed off. The bulb is cleaned, dried, and weighed again and the weight of the sample obtained. This then gives, from the previous measurements of the vapor volume at normal temperature and pressure and liquid volume, the molecular weight and the density of the fraction. In this particular research, the fractions were generally single-component systems, and the molecular weight was sufficient for identification. In some runs mixtures of cyclopentane and 2-pentene were obtained and the densities were used to determine the per cent composition, assuming ideal solutions. For confirmation the liquids were titrated with bromine to determine the olefin content. A discussion of these determinations will appear together with some further data in a later paper.

Table I. Densities and Molecular Weights

Compound	Density	Temperature, ° C.	Molecular Weight
Cyclopentane	0.847	− 98.0	70.1
	0.821	− 80.0	70.1 (theoretical)
	0.802	− 55.0	
	0.792	− 44.0	
2-Pentene	0.752	−105.0	70.2
	0.735	− 80.5	70.1 (theoretical)
	0.720	− 59.5	
1-Pentene	0.751	−105.0	68.6
	0.735	− 80.5	70.1 (theoretical)
	0.718	− 59.0	
	0.711	− 47.0	

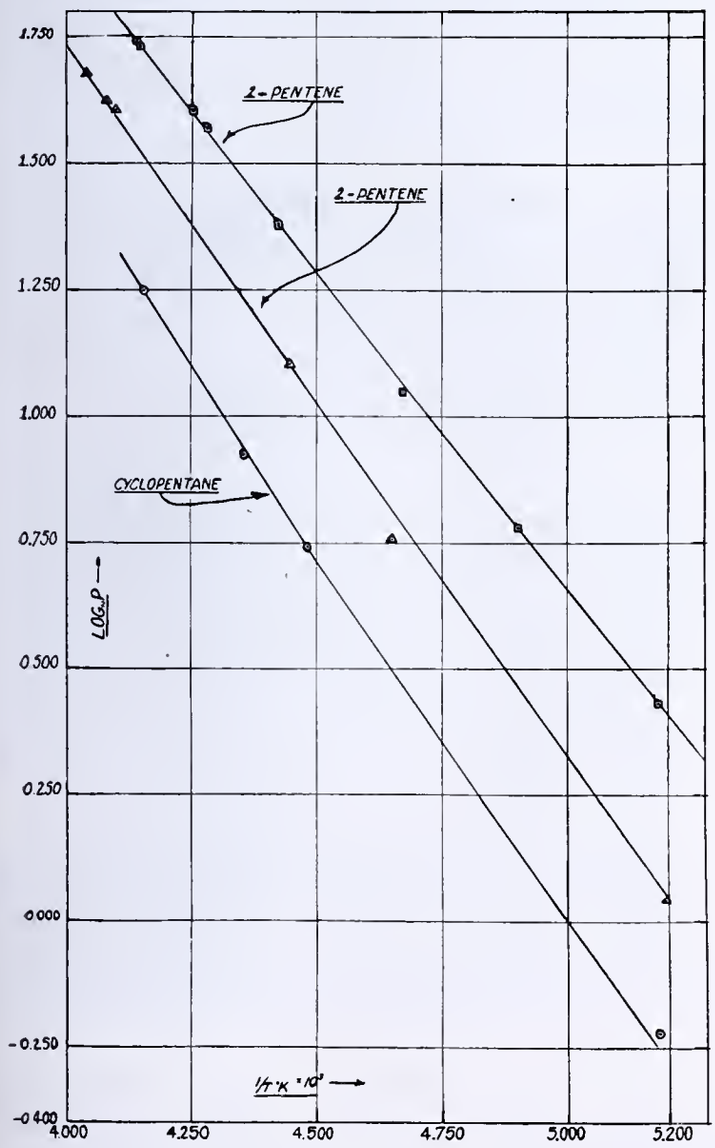

Figure 2. Vapor Pressures

The densities of 2-pentene and 1-pentene are too close to each other to use this property for identification, and in this case the vapor pressures were used to distinguish between them.

The Toepler pump and attached micropycnometer, P_1, are recent additions to enable the measurement of the densities of samples at or near room temperatures. At these temperatures, the vapor pressures are so high that the volume of the other micropycnometer, P_2, is too large and the material at these pressures begins to dissolve in the grease of the stopcock. The presence of stopcock grease in the system can be avoided and the volume of the system reduced to a few tenths of a cubic centimeter by using the Toepler pump as a mercury cutoff. These measurements at room temperature were found expedient, since they made possible the use of the densities noted in the literature. The extrapolation of these densities over a 100° range to the temperature of solid carbon dioxide is too inaccurate.

Materials

For a calibration of the apparatus, measurements were made on samples of cyclopentane, 1-pentene, and 2-pentene. The cyclopentane and 2-pentene were obtained from G. B. Kistiakowsky, who had used them in his calorimetric determinations of the heats of hydrogenation (*6*, *8*). The physical constants of the cyclopentane were: boiling point 49.1° C. at 760 mm. and n_D^{20} 1.4060. The 2-pentene had a boiling point of 36.5° C. at 760 mm. and n_D^{20} of 1.3798.

The 1-pentene was made by the method of Hurd (*7*) and was the middle fraction taken from the fractionation of the main product in a 90-cm. vacuum-jacketed still, packed with glass helices. It had a boiling point of 29.5–31.0° C. at 775 mm. and n_D^{20} of 1.3706.

All these samples were put through several bulb-to-bulb distillations before using them in the system.

Densities and Molecular Weights

The results of the measurements on the density and molecular weight of the hydrocarbons are given in Table I. The quantity of liquid used was in the neighborhood of 20 mg. and was determined to 0.1 mg. In a volume of the system (ca. 200 cc.) it had a pressure of about 30 mm. of mercury.

Table II. Vapor Pressures

P Mm. Hg	Log P	T ° K.	$1/T \times 10^3$
	Cyclopentane		
0.6	−0.222	193.0	5.181
5.5	0.740	223.0	4.484
8.4	0.924	230.0	4.348
17.7	1.248	240.5	4.158
	2-Pentene		
1.1	0.041	192.5	5.195
5.7	0.756	215.0	4.652
12.6	1.100	227.5	4.396
40.3	1.605	244.0	4.098
41.8	1.621	245.0	4.082
47.4	1.676	247.5	4.040
	1-Pentene		
2.7	0.431	193.0	5.181
6.0	0.778	204.0	4.902
11.2	1.049	214.0	4.673
23.9	1.378	226.0	4.425
37.1	1.569	233.5	4.282
40.2	1.604	235.0	4.255
54.0	1.733	241.0	4.149
54.9	1.740	241.5	4.141

The pressure was measured to 0.1 mm. of mercury on a U-tube mercury manometer, with a calibrated, glass, silver-backed scale. The liquid filled the micropycnometer to a height of about 25 mm. and this could be estimated to about 0.2 mm. The diameter of the capillary was about 1 mm. and its cross section about 8.5×10^{-4} cc. per mm. of length. Thus the calculated error on the measurements in Table I is about 0.5 to 1 per cent. This accuracy can readily be improved upon by using a smaller capillary and refining the other measurements—e. g., using a smaller system and weighing on a microbalance to 0.01 mg.

Vapor Pressures

The vapor pressures have been plotted in Figure 2 and the experimental points are recorded in Table II. Over the range of temperatures measured they fit very well the Clausius-Clapeyron equation:

$$\log P = -A/T + B$$

when P is in mm. of mercury at 25° C. and T is in degrees Absolute.

From the slope of the straight lines we can calculate the heats of vaporization in this range of temperature (Table III). In these measurements the pressures were read to 0.1 mm. of mercury and the temperatures to 0.5° C. Care was taken to ensure the establishment of equilibrium which was approached from both higher and lower temperatures. The calculated error in the constants recorded in Table III is about 0.5 to 1 per cent.

Table III. Heats of Vaporization and Clausius-Clapeyron Constants

Compound	A	B	$H_{vap.}$ Kcal./mole
Cyclopentane	1646.0	8.0880	7.524
2-Pentene	1512.5	7.7945	6.913
1-Pentene	1259.5	6.9570	5.757

The measurements of vapor pressures in the case of small samples have necessarily been limited to a narrow range of low pressures, because of the large size of the system. This limits the use of these vapor pressures in the determination of unknowns, since small amounts of volatile impurities introduce a rather sizable error in this range. Furthermore, the pressures are small and therefore not quite so accurate. An apparatus has recently been devised to measure vapor pressures on a micro scale up to the boiling point where small amounts of impurities do not create great errors and where the mole per cent can be determined. This will be fully described in a later paper.

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Electrolytic Deposition of Lead from Biological Material

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IN AN attempt to develop a polarographic method for the determination of microquantities of lead in biological material, it was found that the necessary separation and concentration of the metal could be accomplished electrolytically, provided certain requirements were fulfilled.

In most of the methods reported in the literature for this separation (*3, 4, 9, 10, 13, 14, 15*), lead is first precipitated as the sulfide or oxalate, in order to remove substances which would otherwise interfere with the electrolysis. Any procedure involving precipitation and filtration introduces opportunities for both loss and contamination when microquantities of lead are being handled and therefore a method was sought for the direct electrolysis of the prepared sample. Such a procedure would be even simpler than the one recommended by the Association of Official Agricultural Chemists (*1*), in which the lead is electrolyzed following its extraction with dithizone.

A few methods have been reported in which the lead in biological material is separated as lead dioxide by direct electrolysis of the prepared sample (*5, 8, 12*) or as metallic lead by electrolysis of fresh urine (*7*), but the authors were not able to secure quantitative separations by these procedures. It is possible that failure to obtain a complete recovery of traces of lead escaped notice at the time the methods were devised because precise microanalytical procedures for lead estimation were not then available. These low recoveries are due at least partially to the fact that the electrolyte must be kept acid (pH 3 to 4) in order to prevent precipitation of calcium and lead phosphates, and also to the interference caused in the quantitative deposition of lead by phosphate and iron, which are always present in biological material (illustrated in Table I).

In all experimental data reported in Tables I and II, the quantity of lead deposited on the electrode was determined in the following way:

The lead was stripped from the electrode with about 10 ml. of dilute nitric acid (10 ml. of nitric acid, sp. gr. 1.40, per liter), transferred to a graduated separatory funnel, and 1 ml. of deleaded hydroxylamine hydrochloride solution (20 grams per 100 ml., *2*) was added. The solution was brought to a volume of 50 ml. with the dilute nitric acid and redistilled ammonium hydroxide was added until a pH of 2 was reached (orange color of *m*-cresol purple); from this point the procedure was the same as that previously described (*2*) beginning with "Final Estimation of Lead".

Procedure

The sample is prepared by destroying the organic material at 500° C. as described in previous papers (*6, 11*). The ash is dissolved in nitric and hydrochloric acids and double-distilled water.

Table I. Deposition of Lead

Sample	Pb Present	Lead Found: On anode, electrolyzed by A. O. A. C. method	Lead Found: On cathode, electrolyzed by new method (single)
	Micrograms	*Micrograms*	*Micrograms*
$Pb(NO_3)_2$ solution	50	46	..
$Pb(NO_3)_2$ solution + 0.1 gram of NaH_2PO_4	50	24	..
Same as above + 3 ml. of ammonium citrate solution	50	..	49.5
$Pb(NO_3)_2$ solution + 5 mg. of Fe as $FeCl_3$	50	17	..
Same as above + 3 ml. of ammonium citrate solution	50	..	49.0
Food 2786	22, determined by photometric dithizone method (*2*)	None	..
Same as above + 5 ml. of ammonium citrate solution			22.0

An aliquot or the entire sample, representing 10 to 25 grams of blood, 100 to 250 ml. of urine, 0.1 to 0.15 gram of fecal ash, or $^1/_{20}$ of a day's mixed food, is placed in a 20- to 100-ml. beaker. If the lead on the electrode is to be determined by dithizone, it is not necessary to prevent the deposition of iron, copper, or other substances which might interfere with the determination by other methods. Five to 10 ml. of deleaded ammonium citrate solution (40 grams of citric acid per 100 ml., *2*) are added to the sample, and the mixture is made alkaline to phenol red with distilled ammonium hydroxide, and diluted with 2 to 5 times its volume of double-distilled water.

An electrolytic apparatus similar to that described by the Association of Official Agricultural Chemists (*1*) is employed and the solution is electrolyzed at 5 to 6 volts for 30 to 60 minutes, the cathode serving as the rotating electrode. With the authors' equipment, the current varies from 200 to 500 milliamperes. The lead can then be stripped from the cathode with dilute acid and determined as indicated above. The results of analyses by this method, compared with those obtained by dithizone (*2*) and spectrographic (*6*) procedures, are given in Table II.

If necessary, the electrodes can be washed with 100 ml. of deleaded hydroxylamine hydrochloride solution (0.2 gram per 100 ml.) or 100 ml. of hydroquinone solution (0.1 gram per 100 ml.). This washing should be done by siphoning, so that the electrodes are not exposed to the air at any time, and the electric current should not be shut off while the electrodes are in the solution.

Instead of washing the electrodes, a method of double electrolysis can be employed in which the sample solution is electrolyzed as usual, the cathode is stripped in dilute nitric or hydrochloric acid, 1 ml. of the ammonium citrate solution previously mentioned is added, and the solution (made alkaline to phenol red) is again electrolyzed.

In certain samples, such as those of feces and mixed foods, the copper and iron content is so high as to interfere with subsequent determination of the lead on the electrode by the polarograph. Deposition of those metals, particularly iron, can be prevented largely by the addition of 2 ml. of deleaded potassium cyanide solution (10 grams per 100 ml., *2*) to the solution before electrolysis. This delays the plating action, however, and it is then necessary to electrolyze for 1 to 2 hours. If desired, greater purity of the lead deposit may be secured by double electrolysis with potassium cyanide in each solution, although the deposit is never completely free from certain other metals.

Possible Applications

The method of direct electrolysis, combined with a photometric dithizone procedure for determination of the lead on the electrode similar to that referred to above, provides a rapid and precise method for the determination of lead in biological material. A few modifications of the dithizone procedure would be necessary to make it applicable to routine work.

For instance, instead of three standard dithizone solutions, each suited to a different range of lead concentrations, one solution only could be employed, and varying quantities used according to the amount of lead present. The strength of the solution could be so adjusted that 5 ml. would be equivalent to 10 micrograms of lead; if a 5-ml. portion of the solution turned red after the lead-containing solution was shaken with it, additional quantities of the standard could be used, in 10-ml. portions, until an excess of dithizone was present; the dithizone solution would not be drained out between successive additions, and the color of the final mixture could then be read photometrically. While such a method would not be more accurate than the better dithizone extraction procedures, it would probably be much more rapid, provided a number of electrolytic units were available and could be used simultaneously.

A polarographic method of determining the lead deposited on the cathode will be described in a later paper (*5a*). It is possible that other ways of determining the lead on the electrode, such as titration or colorimetric methods, can also be worked out, particularly in view of the fact that the lead deposit is often very pure.

Summary

In a new method for the electrolytic deposition of traces of lead from biological material, the solution of the ashed sample is electrolyzed directly, without previous separation of the lead. The electrodes can be washed and other metals can be complexed, so that the lead deposit on the cathode is often almost entirely free from other substances. The lead can then be determined by the use of dithizone, the polarograph, or by other methods.

Table II. Deposition of Lead

Sample	Sample Electrolyzed	Lead Found: By electrolysis	Lead Found: By dithizone extraction (*2*)	Lead Found: By spectrograph (*6*)
	Micrograms	*Micrograms*	*Mg./l.*	*Mg./l.*
$Pb(NO_3)_2$	10	9.6[a]	..	..
$Pb(NO_3)_2$	10	9.8[b]	..	..
	Ml.	*Mg./l.*		
Urine 2919	92	0.077	0.077	..
Urine 2919	9.2	0.087	..	..
Urine 3948	66	0.160[a]	0.17	0.17
	Grams	*Mg./100 g.*	*Mg./100 g.*	*Mg./100 g.*
Blood 4397	8	0.067[a]	0.07	0.065
Blood 4011	8.5	0.065[b]	0.055	0.06
Blood 3687	17.5	0.04[b]		0.045
		Mg.	*Mg.*	*Mg.*
Feces 3555	0.1–0.15 ash	0.29[c]	0.30	0.285
Feces 3499	0.1–0.15 ash	0.70[d]	0.71	0.696
Feces 3499	0.1–0.15 ash	0.71[e]	..	..
Feces 3226	0.1–0.15 ash	0.31[d]	0.315	..
Feces 3226	0.1–0.15 ash	0.31[e]	..	..
Food 2786	1/50 day's food	0.022	0.022	..
		Mg./100 g.		*Mg./100 g.*
Tissue (suprarenal) 3894	10	0.057	..	0.045

[a] Electrodes washed with hydroxylamine HCl solution.
[b] Double electrolysis, no KCN present.
[c] KCN present, electrodes washed with hydroxylamine HCl solution.
[d] KCN present, double electrolysis. [e] Single electrolysis, KCN present.

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Microanalytical Determination of Sulfur

A Modified Bomb Method

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THE Elek-Hill microbomb method for the quantitative determination of sulfur in organic compounds (*1*) requires the use of a Neubauer or Gooch type of crucible for the precipitate. The quantitative transfer of microquantities of barium sulfate from a glass dish to a crucible is apt to be attended with some difficulty, owing to the tenacity with which the precipitate sticks to glass. It was thought that if this excellent method of combustion could be modified to permit its use in conjunction with the automatic filtration technique (*6*) a decided advantage would be gained. This change, herein described, is in the nature of a reduction in the amount of fusion mixture, so that the resulting sulfate solution can be evaporated to a small volume and transferred to a filter-stick crucible.

After considerable experimentation this reduction was established, but only after substituting potassium chlorate for the sugar-nitrate mixture used by Elek. The use of potassium chlorate in this connection was also observed by another worker (*3*). The elimination of potassium nitrate reduces the well-known coprecipitation of nitrates, which has been quantitatively studied in a recent paper dealing with macroamounts (*2*). The lowered concentration of salt solution from which the sulfate is precipitated is an added advantage (*4*).

With a mixture of 0.06 gram of potassium chlorate and 0.35 gram of sodium peroxide as the charge, most compounds were quantitatively oxidized. Some substances, particularly sulfones, required treatment with 3 to 4 drops of elementary bromine after the combustion. The bromine treatment is now used as a matter of routine. For this treatment and also to facilitate subsequent transfer of the solution, a 125-ml. "iodine flask" (with a lip for pouring) has been found most convenient.

The procedure is about the same as given by Elek except for the oxidizing mixture given above. The bomb must be heated in a roaring flame for approximately 60 seconds, and in so doing, its lead washer must be protected from excessive heat. This can be accomplished by placing the bomb with its holder in a hole cut out of an ordinary asbestos pad, mounted on a tripod, so that only the cup portion of the bomb is in direct contact with the flame. To acidify the alkaline solution resulting from the combustion 1.5 ml. of concentrated hydrochloric acid are necessary. After the bromine is added to the acidified solution in the flask, the solution is concentrated to about 5 to 7 ml. and transferred to a weighed filter-stick crucible with appropriate rinsings (*5*). The precipitate should be washed thoroughly with a total of about 20 to 30 ml. of 1 to 200 hydrochloric acid and water.

Table I. Typical Analyses

Substance	Sulfur Found %	Sulfur Calcd. %
Cystine	26.74 26.68	26.69
Thiourea	42.01 42.07	42.12
Thiocarbanilide	13.97 14.20	14.05
Sulfanilic acid	18.50 18.57	18.50
Trithioformaldehyde	69.58 69.81	69.59
Sodium β-naphthoquinone sulfonate	12.18 12.39	12.31
Diphenyl sulfone	14.53 14.66	14.68
Diaminophenoxathiin	14.06 14.00	13.92
Glutathione	10.60 10.53	10.42
Sulfonal	27.95 28.09 28.10	28.09
Ethyl-4-methylthiazole-5-acetate HBr	11.95 11.96	12.05
Methionine	21.35 21.43	21.49
P-Br Benzylcysteine	11.12 11.01	11.00
S-Benzyl-*N*-formyl cysteine	13.20 13.36	13.39
Thiamine HCl	9.50 9.46	9.51
Phenyl isothiocyanate (liquid)	23.79 23.68	23.71

Table I gives the results of some typical analyses by the proposed method. The average sample weight was 6.1 mg.; the largest and smallest samples were 9.8 and 3.2 mg., respectively. The average amount of barium sulfate weighed was 8.6 mg.; the largest and smallest precipitates weighed 23.1 and 3.6 mg., respectively.

Since the over-all agreement of these 33 individual analyses with the calculated per cent of sulfur is better than 1 part per 1000 (assuming all the compounds to be pure), it is clear that the effect of coprecipitation is negligible. To check this further, all but one of the precipitates of barium sulfate corresponding to the analyses in Table I were washed again after the initial weighing, reignited, and weighed again (*5*). The average loss in weight was 0.031 mg., distributed as follows:

Loss in Weight *Mg.*	No. of Determinations	Loss in Weight *Mg.*	No. of Determinations
0	1	0.03	12
0.01	1	0.04	9
0.02	6	0.05	3

Since this average loss in weight is less than 4 parts per 1000 of the average weight of barium sulfate obtained in this series, it would appear that coprecipitation does not seriously affect the results.

The reduced amount of fusion mixture obviates the necessity of using reagents of special purity. The regular C. P. grade of sodium peroxide used for macroprocedures comes within the tolerance for this procedure. As Table I indicates, the accuracy of this method is comparable to the other existing determinations. This procedure also has the advantage of speed, especially in multiple runs.

Summary

The reduction of the fusion mixture for the determination of sulfur by the bomb method which, at the same time, permits the use of the filter-stick method for filtration is the basis for the proposed method. The sodium peroxide is reduced from 1.5 to 0.35 gram and 0.06 gram of potassium chlorate is substituted for the 0.30 gram of sugar–potassium nitrate mixture, giving a total of approximately one fifth of the quantity used in the original method.

Acknowledgment

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Modification of Pregl's Apparatus for Electrodeposition

Determination of Copper and Nickel in German Silver

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A modification of Pregl's apparatus for the quantitative microdetermination of metals by electrodeposition permits quantitative collection of electrolyte and washings in an efficient and simple manner. The apparatus was applied to the separation of copper and nickel previous to their determination in German silver. Reasonably satisfactory results were obtained when 5 grams of the alloy were dissolved, and the microanalyses were performed on small portions of the solution. The alloy was not sufficiently homogeneous to give representative samples of 5- to 10-mg. weight when single small shavings were taken for analysis.

PREGL'S apparatus (*7*) was designed for the quantitative microdetermination of copper in preserved foods by electrodeposition and weighing of the metal. No provision had to be made for the quantitative collection of electrolyte and washings. Thus, whenever the electrolyte was needed for further quantitative determinations, a change of design was required (*3*). The simple modification of Pregl's apparatus, which is proposed here, makes use of a siphon (*2*, *5*) for collection of the electrolyte.

Apparatus

The electrolytic cell and the cathode are the same as used by Pregl. The anode consists of a platinum wire approximately 0.5 mm. in diameter, which is wound in the form of a helix around the ascending arm of the siphon (Figure 1). The siphon is made of glass tubing of 2-mm. bore, and two pairs of glass horns are fused to the ascending arm, to hold the anode in the proper position. A small funnel slides up and down on the ascending arm of the siphon and rests on the opening of the cell during electrolysis. The wide test tube with side arm contains a large microbeaker in which the electrolyte and washings are to be collected. The suction tube may be connected with either the vacuum line or a supply of inert gas.

Separation and Determination of Copper and Nickel

The apparatus was applied to the microanalysis of copper-nickel alloys obtained in the form of turnings or shavings for use as students' unknowns. All weighings were performed with an analytical balance, for which the average deviation of a single weighing had been determined as equal to ±0.012 mg.

Approximately 7 mg. of German silver were weighed on a 2.5-cm. (1-inch) watch glass and then transferred into the test tube serving as electrolytic cell. The metal was covered with 50 cu. mm. of distilled water, and a pear-shaped glass bulb, shown in the upper right corner of Figure 1, was placed on the opening of the test tube. The bulb was slightly lifted, and 20 cu. mm. of 16 molar nitric acid were added slowly from a pipet. If necessary, solution was hastened by gently heating with a small flame so that the evolution of nitrogen oxide never became violent. Finally, 10 cu. mm. of 18 molar sulfuric acid were added and heating was continued until solution of the alloy was complete. When the evolution of gases had ceased, bulb and test tube were rinsed down with 0.5 ml. of water, and the bulb was removed. The solution was heated for 5 minutes on a steam bath, and air was blown from time to time into the test tube to remove the liberated nitrogen oxide.

The test tube was now clamped into the electrolytic stand, and the wire gauze cathode was inserted. One drop of ethyl alcohol was added, and then sufficient 1 molar sulfuric acid to bring the meniscus of the electrolyte up to the top of the wire gauze electrode. A slow current of hydrogen was sent through the suction tube and the siphon, and then the siphon with the anode was inserted in the electrolytic cell. The rate of gas flow was immediately adjusted to obtain approximately 1 bubble in 3 seconds emerging from the tip of the siphon touching the bottom of the electrolytic cell.

After making the electric connections (*3*), the electrolyte was heated to approximately 70° C., the flame removed, and the electrolysis started with 2.7 to 3.1 volts across the electrodes. The small funnel shown in Figure 1 must rest on the opening of the cell during the electrolysis. The space between siphon and stem of the funnel was sealed with a drop of water. After 5 minutes of electrolysis the color of the electrolyte indicated that most of the copper had been precipitated. Thus, the electrolyte was heated to boiling and the ring of condensate was driven up close to the opening of the test tube. The flame was then removed, and test tube as well as funnel was rinsed down with a few drops of water. The electrolysis was continued for 10 more minutes, and during the second half of this period the electrolyte was cooled to room temperature, using tap water (*7*). The e. m. f. across the electrodes was never permitted to exceed 3.1 volts.

Without interruption of the current, the electrolyte was completely siphoned off by applying slight suction. The suction was interrupted for washing, and cell and funnel were rinsed with distilled water until the cathode was again completely immersed. The hydrogen current was started and electrolysis allowed to continue for 2 minutes, then the wash liquid was completely siphoned off without interrupting the electric current. The washing was repeated twice in exactly the same manner. Finally, the suction was interrupted, the cathode was again covered with water, and siphon with anode and then the cathode were withdrawn from the cell in the order mentioned. The cathode was prepared for weighing as usual (*7*).

For the determination of nickel, the electrolyte and washings were concentrated on the steam bath to a volume of 1 to 2 ml., and then quantitatively transferred to a microbeaker which previously had been tared together with a filter stick (*4*). The transfer was accomplished by means of a capillary siphon operated by suction. In the microbeaker the solution was treated with 50 cu. mm. of 6 molar hydrochloric acid and 1 mg. of solid ammonium citrate, and mixed by imparting a rotary motion to the contents of the beaker. After addition of a small drop of alcoholic solution of methyl red, air laden with ammonia gas was blown on the surface of the solution in the microbeaker until the color changed to yellow. For this purpose, pure air of low pressure was passed through a gas wash bottle containing dilute ammonia solution (*1*); the contents of the microbeaker were mixed from time to time by a rotary motion with the hand holding the beaker.

The ammoniacal solution remained clear in every instance and was immediately diluted with water to a volume of approximately 4 ml., heated on the steam bath, and treated with a slight excess of alcoholic dimethylglyoxime reagent, which was added in very small drops by means of a reagent pipet with a fine capillary tip. The precipitate collected at the bottom of the

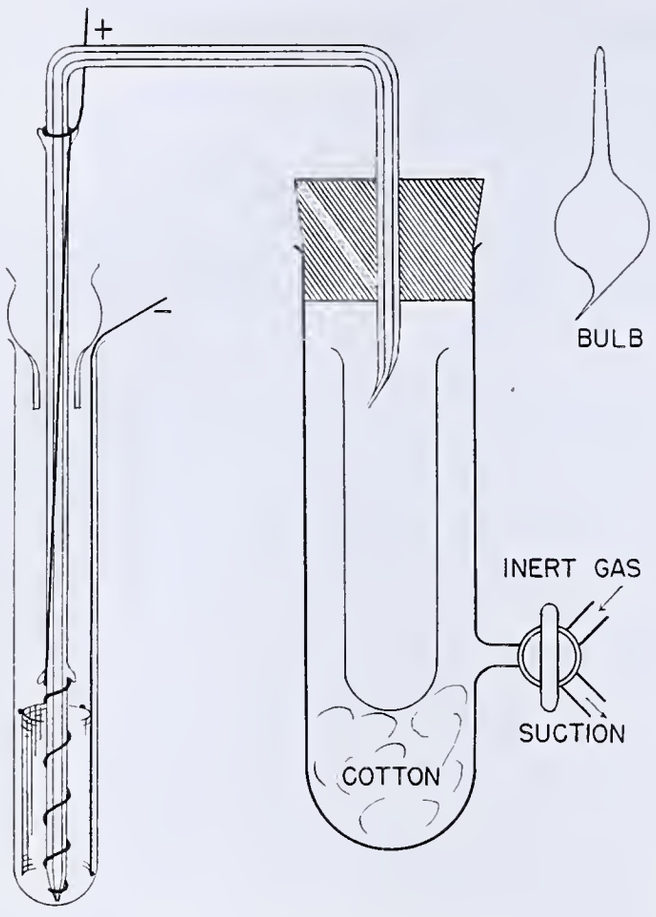

FIGURE 1. DIAGRAM OF APPARATUS

beaker, and it was easy to recognize when the precipitation was complete. Addition of 2 or 3 more droplets of the reagent provided a sufficient excess. The beaker with the precipitate was left for 10 minutes on the steam bath, then placed under a bell jar and allowed to stand for 30 minutes. Filtration (*4*) was followed by washing with four 1-ml. portions of hot water. Each time the walls of the beaker were washed down with hot water from the wash bottle. Beaker and contents were then heated for 1 minute on the steam bath, and the hot liquid was withdrawn through the filter stick. Drying at 120° C. required 10 minutes with the use of the apparatus of Benedetti-Pichler (*4*).

The results presented in Table I show very poor precision, while the averages of the series agree closely with the results of macrodeterminations. To exclude the influence of heterogeneity of the metal shavings, it was decided to repeat the microdeterminations on small aliquots of a solution of a large amount of the alloy.

Thus, 5.007 grams of the German silver were treated in a covered Erlenmeyer flask with 50 ml. of water, 20 ml. of 16 molar nitric acid, and 10 ml. of 18 molar sulfuric acid. Solution of the alloy was effected without heating. The solution was boiled to remove nitrogen oxide, diluted, treated with 1 gram of urea, and boiled again. After cooling, the solution was transferred to a volumetric flask and diluted to a volume of 250.0 ml. After careful mixing, 0.500-ml. portions were transferred to the electrolytic cell by means of a wash-out pipet (*7*). After insertion of the cathode, addition of a drop of alcohol, and dilution with distilled water to immerse the wire gauze electrode, the outline given above was followed without modification.

The results listed in Table II show a considerable improvement of the precision, which is now solely determined by the shortcomings of the chemical procedure, the microtechnique, and the balance used.

TABLE I. DETERMINATIONS ON MILLIGRAM SAMPLES OF GERMAN SILVER[a]

No.	Alloy Taken	Copper Obtained	Nickel Dimethylglyoxime Obtained	Copper	Nickel
	Mg.	*Mg.*	*Mg.*	%	%
1	5.03	3.19	3.91	63.4	15.8
2	6.12	4.10	5.11	67.0	16.9
3	4.76	2.95	..	62.0	....
4	4.62	2.87	4.01	62.1	17.5
5	4.16	2.44	3.31	58.7	16.2
6	4.19	2.59	3.31	61.8	16.1
7	9.94	4.46	7.52	62.2	15.4
8	8.74	5.37	6.82	61.4	15.9
			Arithmetical mean	62.3	16.2
			a_s	±1.4	±0.55
			Macroanalysis[a]	62.58	16.20

[a] Supplied by The Fales Chemical Co., Inc.

The spray produced by the liberation of gases from solutions constitutes a very serious source of loss in microprocedures. One must, therefore, carefully guard against such losses during the process of solution. Addition of alcohol to the electrolyte serves to reduce the stability of the very fine spray obtained during electrolysis; nevertheless, part of this spray would be carried out of the electrolytic cell, if too rapid a current of inert gas were passed through the electrolyte. The current of inert gas is necessary to prevent electrolyte from entering the siphon before precipitation of copper is complete. The stirring effect (*6*) must not be overrated, since the gas current has to be slow, for the reasons mentioned.

TABLE II. DETERMINATIONS ON 0.500-ML. PORTIONS OF A SOLUTION OF 5.007 GRAMS OF GERMAN SILVER IN 250.0 ML.

No.	Copper Obtained	Nickel Dimethylglyoxime Obtained	Copper	Nickel
	Mg.	*Mg.*	%	%
1	6.24	7.75	62.21	15.6
2	6.25	7.83	62.31	15.9
3	6.29	7.72	62.71	15.6
4	6.27	7.82	62.51	15.8
5	6.25	8.04	62.31	16.3
6	6.25	8.03	62.31	16.3
7	6.26	8.04	62.46	16.3
		Arithmetical mean	62.40	16.0
		a_s	±0.14	±0.26
		Macroanalysis[a]	62.58	16.20

[a] Supplied by The Fales Chemical Co., Inc.

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Bromo Complexes for the Identification of Metals and Alkaloids

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Conditions are described for the use of bromometallic salts of alkaloids, sparingly soluble in bromide solution, as microanalytical tests for the metals of the mercury, cadmium, bismuth, antimony, lead, and tin group, or as tests for alkaloids of certain tertiary types. Such salts are often beautifully crystalline and characteristic in appearance. Likely sources of interference are discussed, and concentration limits are given for alkaloids and for metals. Several reactions of special significance are indicated. Application of the tests to mixtures has not been studied.

THE formation and properties of salts of alkaloids with metallic bromo complexes, salts which are sparingly soluble in bromide solutions, have been described (*4*). The composition of selected salts is there shown to conform to the formulas B_xMBr_4 or B_xMBr_5, where B represents an alkaloid ion and M a metallic ion, divalent in the first formula and trivalent in the second. Of the common metals only the bromo complexes of mercury, cadmium, bismuth, antimony, lead, and tin give difficultly soluble salts with alkaloids. The ability of alkaloids to give sparingly soluble salts of this kind depends upon the type of nitrogen in the organic base, and it has been demonstrated that only certain types of tertiary bases possess this property. Thus, tests with metallic bromo complexes may be of use in elucidating the type of nitrogen, and the behavior of alkaloids of known constitution may be predicted within certain limits.

The present paper deals with the use of bromo complexes in microchemical tests for alkaloids and for the above-mentioned small group of metals. The test for cadmium based on the separation of the brucine salt of the bromo complex was introduced by Meurice (*3*) and later used as a slide test by Martini (*1*), who (*2*) also employed the bromo complex of lead for the microchemical detection of cocaine and other alkaloids. Recently the use of the bromo complexes of cadmium, mercury, gold, and platinum for the detection of alkaloids has been described by Whitmore and Wood (*5*), but the work reported below had been completed without knowledge of their paper. Certain discrepancies between their results and those of the writer have been checked carefully. They appear to be caused by differences in the techniques and concentrations.

Tests for Metals

One drop, 0.015 ml., of the solution of the metal is placed in the depression of a hollow-ground slide, and to it are added 2 drops of a 40 per cent solution of alkaloid in either water or 2 per cent acid. Small amounts of acid, up to 10 per cent in the test drop, have little effect on the sensitivity of the tests. The test is observed under the low-power microscope for 20 minutes. This time suffices for the crystallization of amorphous precipitates and for the separation of crystals from very dilute solutions.

The exact shape and arrangement of crystals are reproducible over a large range of concentrations. Some typical precipitates are shown in photomicrographs (Figures 1 and 2) of bromometallic salts of alkaloids formed by 1 drop of 1 per cent metal solution, 2 drops of 40 per cent potassium bromide solution, and 1 drop of 1 per cent alkaloid solution. No attempt is made to describe the appearance of precipitates, since in practice it will be best to compare the forms with those obtained from known solutions of approximately identical concentration. Complications may arise from the separation of difficultly soluble metal bromides, alkaloid hydrobromides, and products of the hydrolysis of metallic ions. Silver bromide is practically insoluble in potassium bromide solution. If much lead is present, the 2 drops of bromide solution are insufficient for the complete solution of the lead salt. The formation of crystalline hydrobromides of certain alkaloids is discussed below. The oxyhalides of bismuth and antimony may be dissolved by adding more acid which, of course, causes some reduction of the sensitivity of the tests. The statements in Table I concerning the crystalline or amorphous appearance of the precipitates apply to the use of a 1 per cent solution of the metallic ion. Use of the following alkaloids may be generally recommended:

Cocaine. Presence of lead causes the separation of needles (Figure 2, 11) which are readily detectable in the presence of large amounts of amorphous precipitate produced by mercury, cadmium, antimony, or bismuth. The concentrations of tin normally met with are not liable to interfere with this very sensitive test for lead.

Tropine. Only lead gives a precipitate (Figure 2, 12) not specially sensitive.

Brucine. All six metals mentioned above are precipitated even in dilute solution. In spite of the high limiting concentration for tin, brucine may be used as a group reagent to prove the absence or presence of mercury, cadmium, antimony, bismuth, lead, and tin. The separation of small amounts of brucine hydrobromide, the crystals of which resemble those obtained with mercury or cadmium, must be prevented by adding a small amount of dilute acid.

The crystals formed with cadmium (Figure 1, 1) and mercury (Figure 1, 2) are similar in appearance, and so are those with bismuth (Figure 1, 3), and antimony (Figure 1, 4). Lead produces an amorphous precipitate. Since tin does not give a precipitate

Table I. Limiting Concentrations for Metals

	Hg	Cd	Sb	Bi	Pb	Sn
Narcotine	3000 A[a]	2000 A	700 A	700 A	300 A	..
Narceine	900 A	100 A	...	200 A	...	..
Cotarnine	3000 C	4000 C	200 A	200 A	300 C	..
Hydrastinine	800 C	800 C	100 A	100 A	50 C	..
Morphine	40 A	...	...	...	...	..
Codeine	1000 C	200 C	...	...	...	..
Dionine	20 A	20 A	...	20 A	...	..
Apomorphine	100 A	...	100 A	...	...	..
Quinine	300 C	100 C	80 A	30 A	...	..
Quinidine	....	....	....	....	....	..
Cinchonine	1100 C	700 C	100 A	100 A	...	..
Cinchonidine	....	....	...	...	...	..
Sparteine	300 C	100 C	....	....	....	..
Brucine	4000 C	3500 C	11000 C	30000 C	700 A	10 A
Yohimbine	350 A	100 A	100 A	100 A	...	..
Aconitine	800 A	150 A	...	...	....	..
Tropine	....	....	....	...	600 C	..
Cocaine	800 A	300 A	100 A	500 A	25000 C	50 C
Tropacocaine	6000 C	9000 C	150 A	2000 A	1000 C	..
Atropine	...	...	...	...	30 C	..

[a] A, amorphous; C, crystalline.

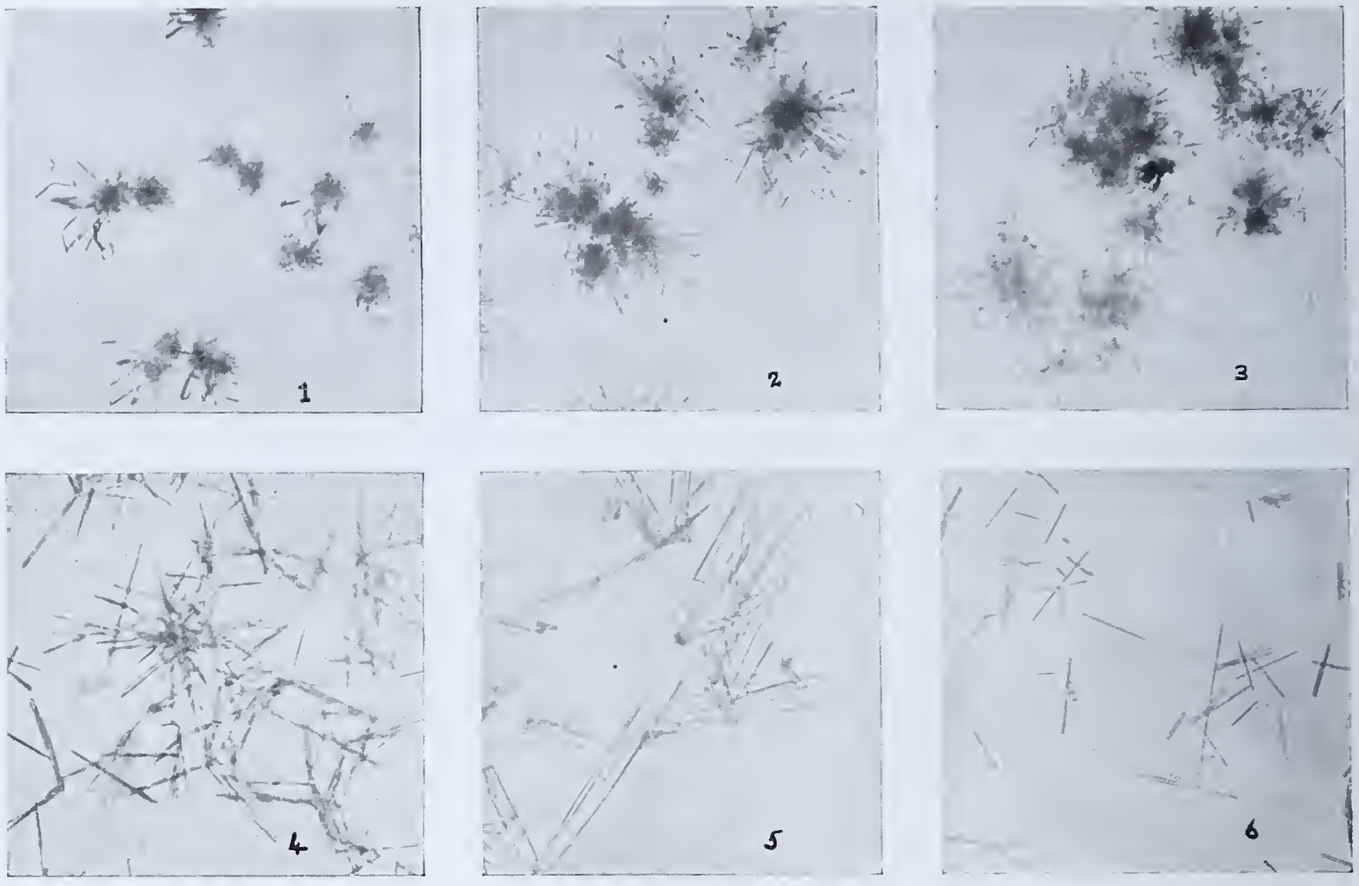

FIGURE 1. BROMOMETALLIC SALTS OF ALKALOIDS (× 100)

1. Cadmium brucine bromide
2. Mercury brucine bromide
3. Bismuth brucine bromide
4. Antimony brucine bromide
5. Cadmium cotarnine bromide
6. Mercury cotarnine bromide

at moderate dilution, brucine may be used for the detection of antimony in a not too strongly acid solution of Group II B of the analytical scheme. The brucine reagent is not recommended as a test for cadmium in the presence of copper, since other reagents of better selectivity and higher sensitivity are available for this purpose.

COTARNINE. Crystals separate with cadmium, mercury, lead, and bismuth (Figures 1 and 2). Antimony gives an amorphous precipitate.

Tests for Alkaloids

The tests are carried out in the same manner as for metals. One per cent solutions of the metals are first treated with twice the volume of bromide solution, and the solution of alkaloid is then added to the mixture. The alkaloids are dissolved in water or 2 per cent acid, so as to obtain solutions of the concentration range between 1 in 50 and 1 in 100. This concentration range is necessary, since with dilute solutions of the alkaloid often no precipitates are obtained, as may be seen from Table II.

TABLE II. LIMITING CONCENTRATIONS FOR ALKALOIDS

	Hg	Cd	Sb	Bi	Pb
Narcotine	300 A	200 A	200 A	300 A	100 A
Narceine	300 A	100 A	...	200 A	...
Cotarnine	800 C	900 C	200 A	200 C[a]	400 C
Hydrastinine	400 C	300 C	200 A	200 A	400 C
Morphine[b]	30 A	...	...	...	...
Codeine	300 C	300 C	...	...	...
Dionine	20 C	20 A	...	20 C	...
Apomorphine	100 A	...	200 A	...	...
Quinine	150 C	100 C	80 A	30 A	...
Quinidine	40 A	30 A	40 A	80 A	...
Cinchonine	300 C	250 C	20 A	100 A	...
Cinchonidine	30 A	30 A	20 A	30 A	...
Sparteine	200 C	100 C	...	...	...
Brucine	450 C	350 C	2000 C	3000 C	150 A
Yohimbine	250 A	...	...	...	...
Veratrine	200 A	200 A	100 A	100 A	100 C
Aconitine	250 A	200 A	...	...	...
Tropine	...	...	...	...	300 C
Cocaine	150 A	100 A	100 A	150 A	550 C
Tropacocaine	2500 C	1000 C	1200 C	750 A	1000 C

[a] Very small C.
[b] Gelatinous, some aggregation to an ordered arrangement.

The notations "crystalline" and "amorphous" (C and A, Table II) are based on the above-described conditions of the test. The effect of the concentration on the appearance of the precipitate becomes significant with either very concentrated or very dilute solutions. From concentrated solutions precipitates described as crystalline may occasionally separate in an amorphous state and crystallize slowly. Some amorphous precipitates may be crystallized from hot water. The appearance of the precipitates should be compared under the microscope with that of precipitates obtained from known solutions of approximately identical concentrations. The possibility of a separation of oxybromides and of alkaloid hydrobromides must be continuously kept in mind.

With berberine, emetine, harmine, and strychnine the hydrobromides precipitate profusely under the conditions of the test, using 1 per cent solutions of the alkaloid. Other alkaloids, like brucine, codeine, and tropacocaine, give only a few crystals of hydrobromide under these conditions. The trace of brucine hydrobromide is similar in appearance to the crystals produced by the cadmium or mercury reagent in dilute solutions of the base. The interference of the hydrobromide is negligible, however, if the brucine is applied in 5 per cent acid solution. With codeine the separation of the hydrobromide affects the identification of the bromo salts, especially when the conditions of the test approach the low concentrations. Limiting concentrations given in Table II are those at which the bromo salts can be detected readily in the presence of hydrobromide. The hydrobromide of tropacocaine separates in thin plates exhibiting interference colors. By

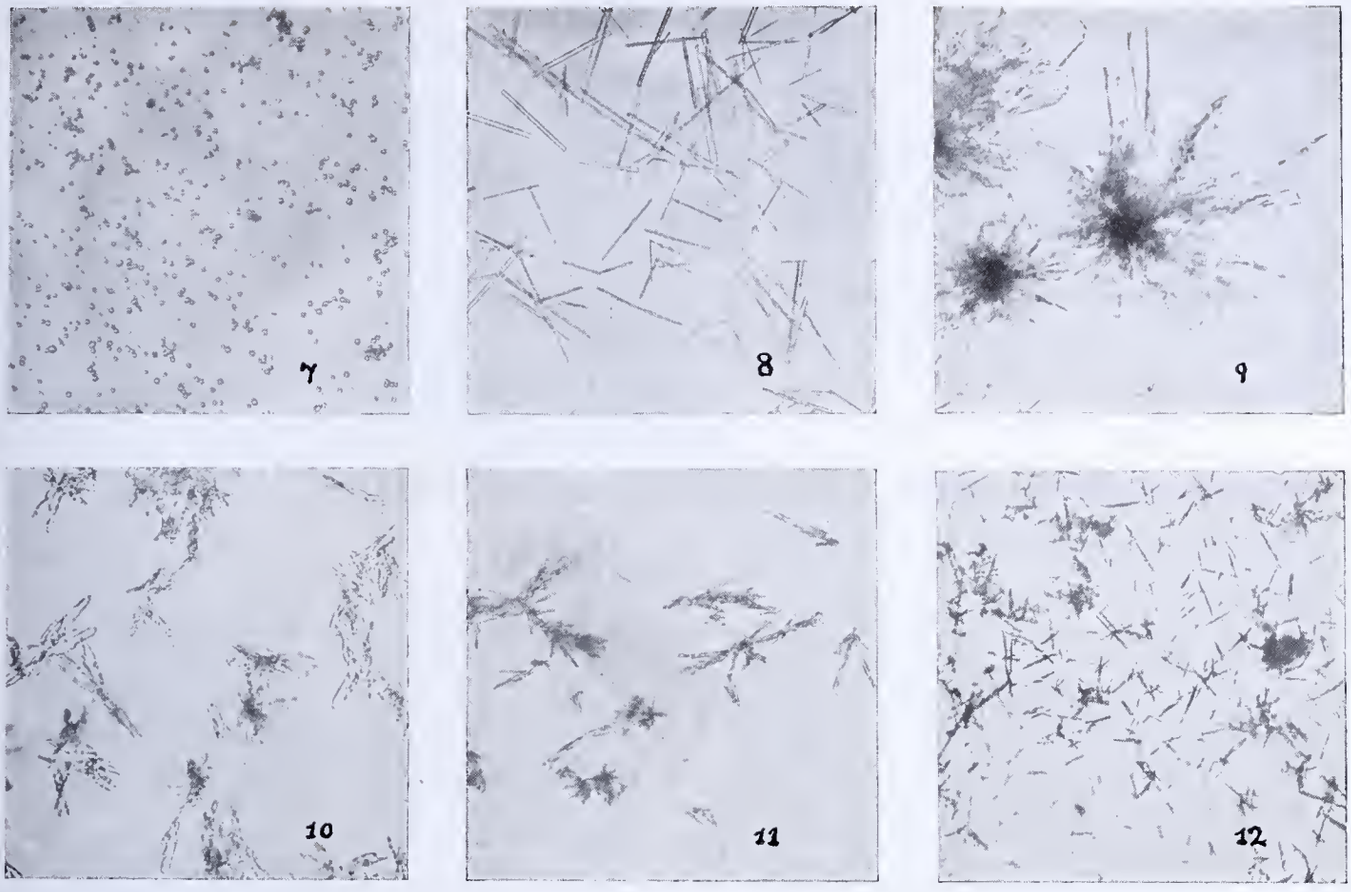

FIGURE 2. BROMOMETALLIC SALTS OF ALKALOIDS (× 100)

7. Bismuth cotarnine bromide
8. Lead cotarnine bromide
9. Cadmium cinchonine bromide
10. Lead tropacocaine bromide
11. Lead cocaine bromide
12. Lead tropine bromide

using a 10 per cent solution of potassium bromide, the separation of the hydrobromide can be delayed for a long time and the bromo salts can be identified readily. No difficulty should be experienced with certain other alkaloids giving hydrobromides crystallizing sparingly only when 5 per cent solutions of the base are employed: aconitine, dionine, narceine, narcotine, and yohimbine. Concentrated solutions of cinchonidine and quinidine produce oily drops which crystallize on adding acid.

A few organic bases give precipitates of different structure from the bromo salts, soluble in an excess of bromide solution. Papaverine and ethylenediamine are examples (*4*). Some of the precipitates listed in Table II are slightly soluble in bromide excess, and the limiting concentrations can be improved in such instances by the use of less potassium bromide. This holds for dionine among others.

The bromo complex of tin is not suited as an alkaloid reagent, tin giving no precipitates in 1 per cent solution (Table I). Using the bromo complexes of mercury, cadmium, antimony, bismuth, and lead, the following scheme is suggested for the identification of alkaloids.

Identification of Alkaloids

I. Sparingly soluble hydrobromides
  A. A large precipitate is given by 1 per cent solutions of the alkaloid: berberine C, emetine A, harmine C, strychnine C
  B. Large precipitates form only from more concentrated solutions of the alkaloids: aconitine C, brucine C, codeine C, narceine C, narcotine C, tropacocaine C, yohimbine A
  C. Oily globules separate which crystallize on adding acid: cinchonidine, quinidine

II. Number of bromo complexes giving precipitates
  Five. All A: narcotine
    Lead C, others A: cocaine, veratrine
    Lead, mercury, cadmium C, others A: hydrastinine, tropacocaine
    Lead A, others C: brucine
    Antimony A, others C: cotarnine
  Four. Mercury, cadmium, bismuth, antimony A: cinchonidine, quinidine
    Mercury, cadmium C, bismuth, antimony A: cinchonine (Figure 2, 9), quinine
  Three. Mercury, cadmium, bismuth A: narceine
  Two. Mercury, cadmium C: codeine, sparteine
    Mercury C, cadmium A: dionine
    Mercury, cadmium A: aconitine, apomorphine
  One. Mercury A: morphine, yohimbine
    Lead C: tropine
  None (up to 5 per cent solutions of the alkaloid). Adrenaline, arecoline, atropine, caffeine, colchicine, coniine, cytisine, ephedrine, eserine (physostigmine), homatropine, hyoscyamine, hyoscine (scopolamine), *d*-lupanine, nicotine, taxine, and theobromine

III. Bromo complexes of mercury and bismuth
  Both C: brucine (Figure 1, 2 and 3), cotarnine (Figures 1 and 2, 6 and 7), dionine
  Mercury C, bismuth A: cinchonine, hydrastinine, quinine, tropacocaine

IV. One bromo complex
  Cadmium, no precipitate: apomorphine, morphine, tropine, yohimbine
  Mercury, no precipitate: tropine
  Bismuth C: brucine (Figure 1, 3), cotarnine (Figure 2, 7), dionine
  Antimony C: brucine (Figure 1, 4)
  Lead C: cocaine (Figure 2, 11), cotarnine (Figure 2, 8), hydrastinine, tropacocaine (Figure 2, 10), tropine (Figure 2, 12), veratrine
  Lead A: brucine, narcotine

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MODERN LABORATORIES

New Science Building at Pennsylvania State College

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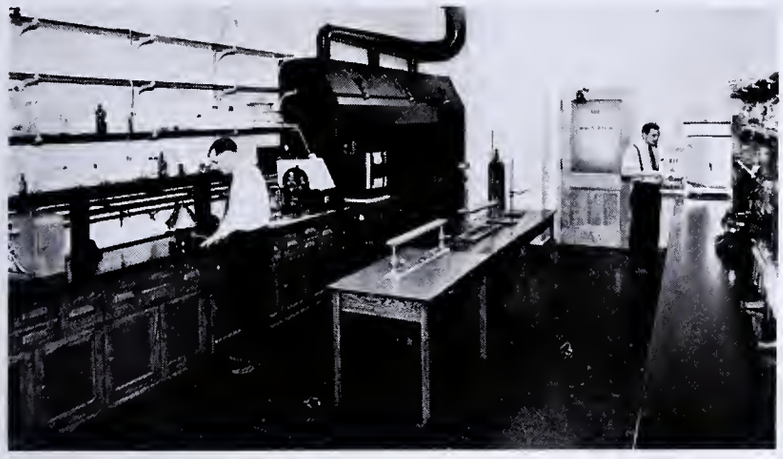
THE Departments of Agricultural and Biological Chemistry and Zoology and Economic Entomology of Pennsylvania State College are now occupying the new Agricultural Science Building, one of the several new buildings made available by the General State Authority in 1940. This brief description deals solely with the facilities of the Department of Agricultural and Biological Chemistry.

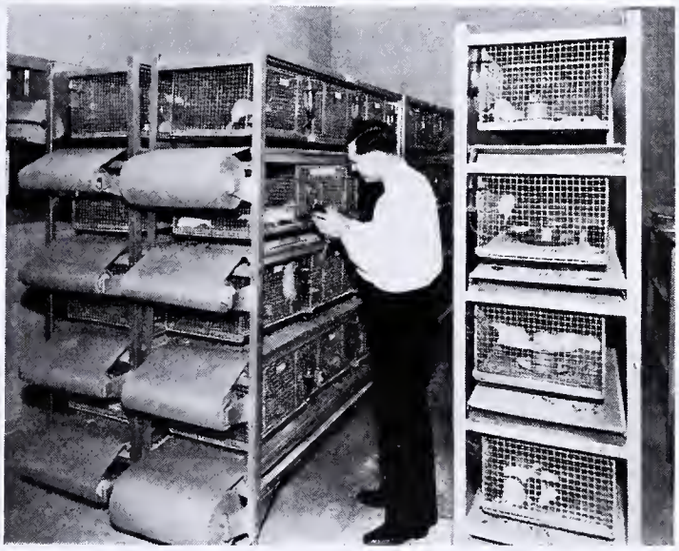
The building has been named Frear Laboratories in honor of the late William Frear, nationally known agricultural chemist, who devoted 37 years to Pennsylvania agriculture and national pure food problems. It is constructed of cream-colored Roman brick, trimmed with Indiana limestone. The building measures 205 by 70 feet and consists of four floors. Owing to the contour of the campus three floors are above ground level on the front, while four floors are above ground at the rear.

All laboratories are well lighted and have cream-colored tile walls equipped with adjustable shelving on aluminum brackets set in aluminum slots. This makes it possible to have shelves of any desired width or height without mutilating the walls. Acidproof plumbing is standard equipment throughout and all plumbing is exposed (overhead) for convenience in making repairs.

Floors, with the exception of a few special rooms, consist

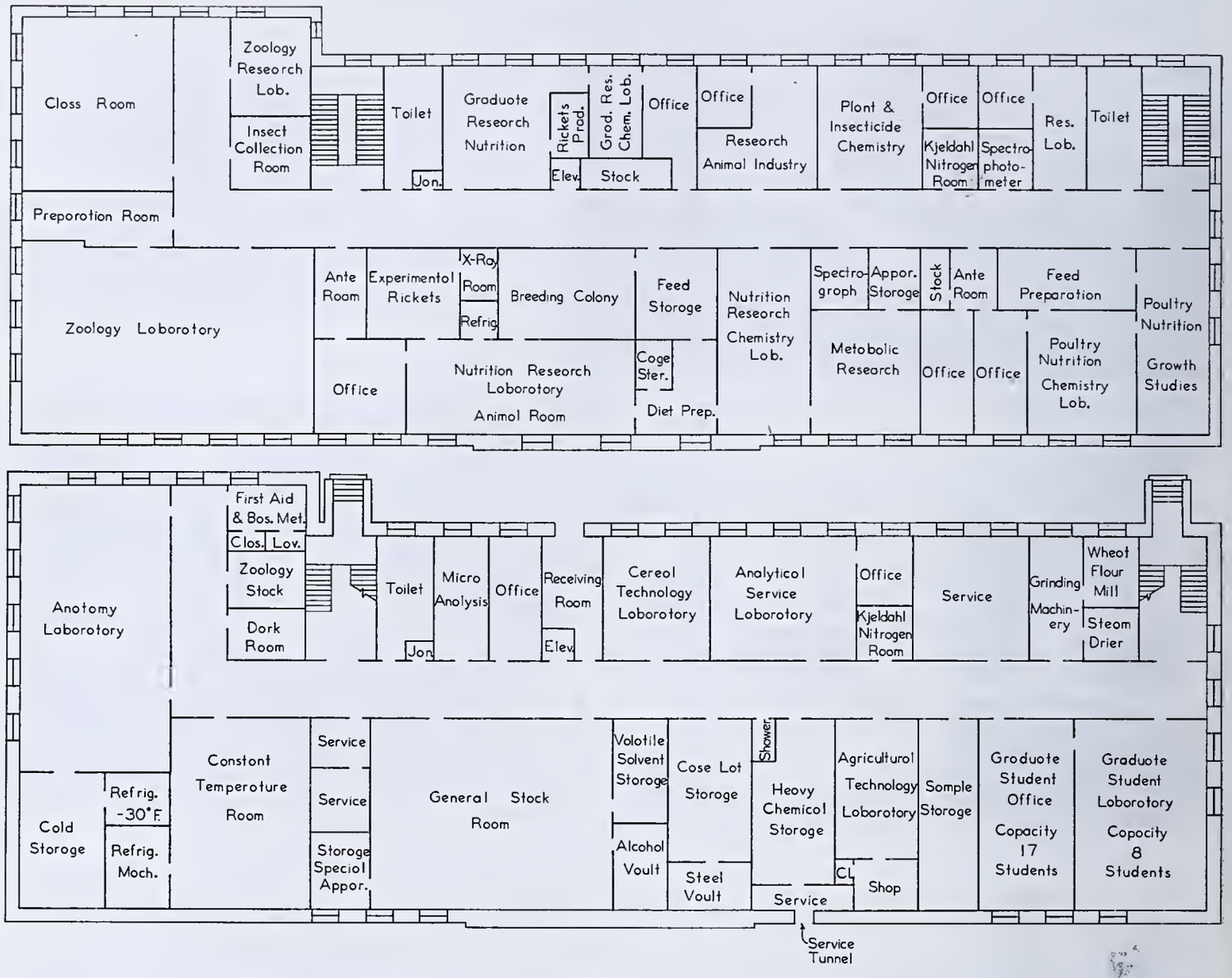
Upper Left. Corner of Protein Research Laboratory
Lower Left. Graduate Student Laboratory, Accommodating Eight Students
Upper Right. Typical Student Laboratory
Lower Right. Private Laboratory for Tobacco Research





UPPER LEFT. LABORATORY FOR VITAMIN RESEARCH
LEFT CENTER. CEREAL TECHNOLOGY LABORATORY
LOWER LEFT. LABORATORY FOR RESEARCH ON ANIMAL PRODUCTION PROBLEMS
UPPER RIGHT. POSTGRADUATE WORK IN BIOPHYSICAL CHEMISTRY
RIGHT CENTER. AIR-CONDITIONED BREEDING ROOM, ANIMAL LABORATORY
LOWER RIGHT. AIR-CONDITIONED CAGE ROOM FOR VITAMIN RESEARCH

of black linoleum tile. All laboratory desks are of wood construction with tops made of chemically treated laminated pressed wood, covered with a chemical-proof plastic finish. Desks in all laboratories are equipped with gas, cold and hot water, compressed air, vacuum lines, steam, and alternating and direct current Distilled water is conducted in aluminum tubing to laboratories and corridors from reservoirs in the attic. Forced ventilation is available for all classrooms and laboratories and all fume hoods are equipped with individual motors. Four large student laboratories, each of which will accommodate from 192 to 224 students, are designed for the various service courses in biochemistry. Balance, Kjeldahl, fat-extraction, and instrument rooms are sepa-



ABOVE, THIRD FLOOR; BELOW, BASEMENT

rate from but easily accessible to the large student laboratories, as are three small classrooms, with a seating capacity of from 45 to 60. Two laboratories are set aside for graduate student research and a large office accommodates from 15 to 20 graduate students. Other graduate students have offices adjacent to private laboratories of professors with whom they are working on special problems.

Two cold rooms, maintained at 32° F., are available for quantity storage, while all laboratories are equipped with electric refrigerators for active laboratory materials. One room, for low-temperature work, is kept at −20° F. but can be used at temperatures as low as −32° F. A laboratory is also designed for pilot-plant and semicommercial-scale research in agricultural technology.

Milling facilities are available for grinding samples of every type, from rocks and soils to foods and feeds. An experimental flour mill is available for work in cereal technology. A specially designed steam dryer, made of stainless steel, has a capacity of 200 to 400 pounds for rapid drying of such materials as silage, tobacco, and agricultural crops.

A complete baking laboratory has been designed for student instruction and research. This laboratory has a baking oven with rotary hearth, fermentation cabinet (automatically controlled for temperature and humidity), dough mixers, sheeting roll, molder, and complete equipment for measuring gluten strength of flours, loaf volume, etc.

All principal storage rooms are on the basement floor. Small subsidiary stockrooms (for dispensing of stock) are located on each laboratory floor. Supplies are carried to the subsidiary stockrooms by elevator.

Experiment Station Projects

The top floor is devoted solely to research work on experiment station projects. Instrument rooms containing quartz spectrograph, spectrophotometer, fluorometers, polariscopes, and colorimeters, and an x-ray room and a darkroom for photographic work are available for all workers. Air-conditioned laboratories have been designed for animal nutrition studies and facilities are available for sterilizing all cage equipment. Battery cages are used for poultry nutrition studies and separate laboratories are available for student research in nutrition. Observation windows have been constructed so that the public can see the animals in the breeding colony and in the nutrition laboratory without entering the air-conditioned rooms and interfering with the regular research work.

The Department of Agricultural and Biological Chemistry has 108 undergraduate students majoring in the department and 33 candidates are now working for advanced degrees. The department offers service courses to approximately 1200 students from other departments and schools on the campus.

INDUSTRIAL AND ENGINEERING CHEMISTRY

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Mineral Oil Deterioration System

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THE application of mineral oils for various service applications, such as for electrical insulation and for lubrication, depends upon an interpretation of the initial properties or characteristics of the oil in terms of the deterioration characteristics in service. Such interpretation depends upon fundamental studies, appropriate deterioration tests, and service records. The general qualities of mineral oils for insulation, lubrication, and other uses can to some extent be determined by subjecting the oil to short-time aging tests, although these tests do not fully represent long-time service conditions. Provided the results can be properly interpreted, short-time tests which last for a few days or weeks are to be preferred to service tests which may continue for years. Further, artificial deterioration under laboratory conditions, where there is more flexibility and better control of the variables involved, gives fundamental information about the deterioration mechanism which is difficult to obtain from other tests.

The most common method of deteriorating insulating oils is to oxidize the oil either alone or in the presence of other materials, the oxidation being carried out at an elevated temperature to increase its rate. A modified aging treatment is to heat the oil in an inert atmosphere for an extended time, either alone or in the presence of other substances. A combination of these treatments, which approximates conditions in practice, consists in oxidizing the oil until a definite quantity of oxygen has reacted and then continuing the deterioration by heating in the absence of oxygen. As catalysts, the materials added may be powdered or bulk metal oxides such as copper and lead oxides, soaps such as copper naphthenate or stearate, or pure metals such as copper (wire or cubes). Paper, which absorbs some of the oxidation products and may possibly be a source of oxygen, may be added in aging processes to approximate conditions in a cable. Other aging treatments consist in subjecting the oil to bombardment or exposing it to ultraviolet light. A different approach to the cable problem consists in aging short lengths of cable by subjecting them to various conditions of temperature and voltage.

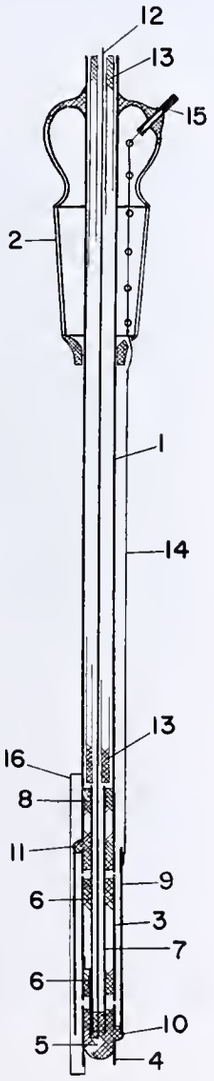

FIGURE 1. ELECTRICAL MEASURING CELL

However, in the authors' laboratory aging tests have been made using oxygen or nitrogen (or both), with and without catalysts. The deterioration of an oil is accompanied by the liberation of volatile products, the addition of oxidation products to the oil, and changes in the physical and electrical properties. Since the chemical and physical changes which occur give a measure of the progress of the deterioration, the ideal procedure would be to follow accurately and completely all the changes which occur; but this at present is neither possible nor practicable. Ordinarily a definite property or set of properties is measured as the deterioration progresses. The properties selected are dictated in many cases by limitations of the apparatus used, and to a large extent by the field of application of the oil. In order that the limitations imposed by the apparatus may be eliminated as far as practicable, special attention has been given to the design of systems for deteriorating mineral oils by oxidation and heating. The features that have been sought are given here:

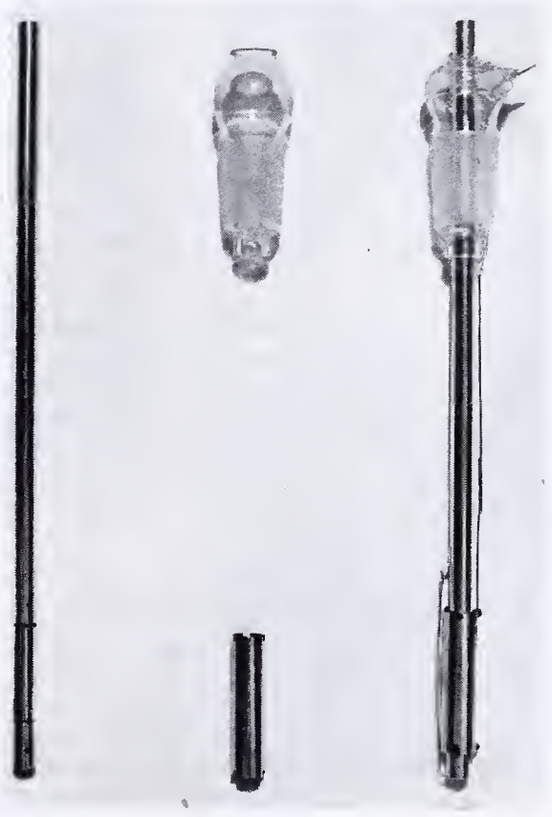

FIGURE 2. THREE-TERMINAL PLATINUM-GLASS ELECTRICAL MEASURING CELL

An all-glass apparatus
A closed system
An adequate dispersion of the gas phase in the oil and an adequate circulation of the liquid phase
A system which permits oxygen absorption measurements to be made
A method of withdrawing samples at intervals
A means for adding catalysts and other materials
Provision for removing or retaining volatile products as desired
The ability to make electrical measurements on the sample in the deteriorating system
A system which will permit making studies on small volumes of oil, so as to make practicable work with straight hydrocarbons or special samples that may be taken from various stages of a refining process

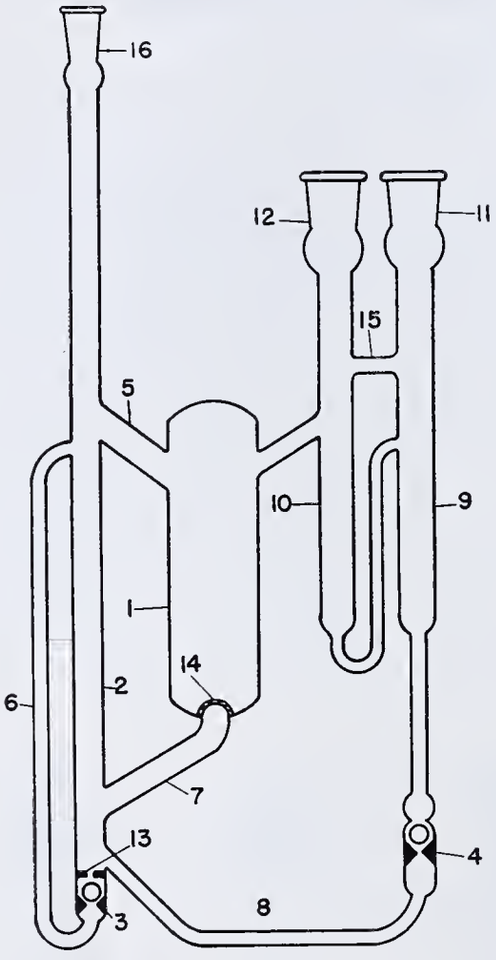

FIGURE 3. DETERIORATION CELL

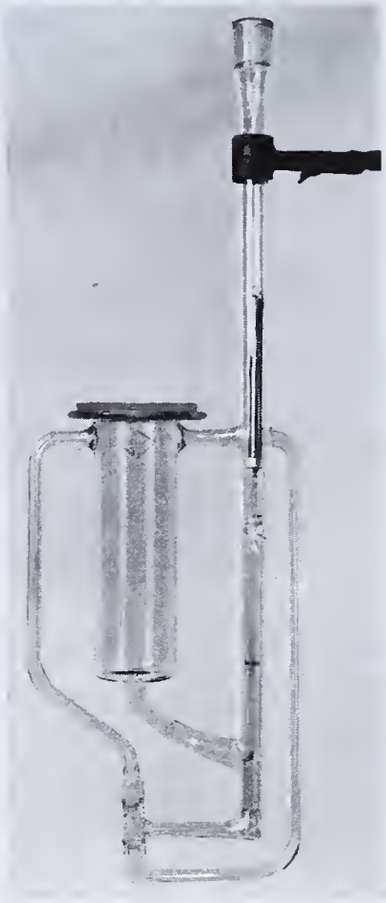

FIGURE 4. DETERIORATION CELL

FIGURE 5. DETERIORATION CELL WITH ELECTRICAL MEASURING CELLS

The quantity of oxygen absorbed in a unit weight of oil is considered to be the independent variable in an oxidation study, although the time during which the oil is subjected to oxidation may be used as that variable. The latter quantity must be considered, but is not sufficient in itself, because the rate at which oxygen is absorbed by an oil varies with time for a given oil and the rate of absorption as a function of time is greatly different for different oils.

Electrical Measuring Cells

The electrical measuring cell (*1*) is shown schematically in Figure 1 and in assembled form in Figure 2. The cells are also shown as used in the deterioration cells for electrical measurements during a deterioration test in Figures 5 and 6. These cells are utilized for making such measurements as conductivity, loss factor, and dielectric constant of the oil and an oil-impregnated paper sample at intervals during the deterioration.

The cell is of the three-electrode type for precise measurements and is of platinum-glass construction to give a minimum electrode effect on the sample, and to permit effective cleaning.

The principal parts of the cell as indicated in Figure 1 are: a guard section, tube 1, 6.25 mm. (0.250 inch) in outside diameter by 0.175 mm. (0.007 inch) wall, sealed to a No. 15 ground joint, 2, through a graded seal; a measuring section, tube 3, and guard section, tube 4, each 0.250 inch in outside diameter by 0.007 inch wall supported by glass seals 5 and 6 from tube 7, this tube being supported from the assembly supporting guard tube 1 by glass seal 8; a high-voltage section, tube 9, 9.1 mm. (0.364 inch) in outside diameter by 0.007 inch wall supported by three glass pillars, 10 and 11, at each end from guard tubes 1 and 4.

Electrical connections external to the cell are made with 20-mil platinum wire spot-welded to the appropriate electrodes; guarded lead 12 with glass spacer 13 for connection to measuring section 3 and supporting tube 7; high-voltage lead 14 sealed in ground joint at 15; guard connection is made directly to guard supporting tube 1 external to the cell with guard tubes 1 and 4 connected together by lead 16 within the cell.

While different relative dimensions may be used in the construction of a cell of the type described, the cells normally used have been made with a measuring section length of 2.5 cm. (1 inch) and a 50-mil spacing, giving a vacuum capacitance of approximately 4.1$\mu\mu$f. The length of the cell shown is determined principally by the size of the deterioration cell in which it is used and the maintenance of the ground-glass joints external to the oven in which the deterioration cell is placed. The size of the cell may be decreased from that shown and described, and can be constructed so that the oil volume required for a measurement is principally that contained between the measuring electrodes. Thus, it may be adopted to measuring small volumes of oil, the present cell requiring only 3 cc.

Deterioration Cells

The deterioration cells for containing the oil sample and other material such as copper and paper, and the electrical

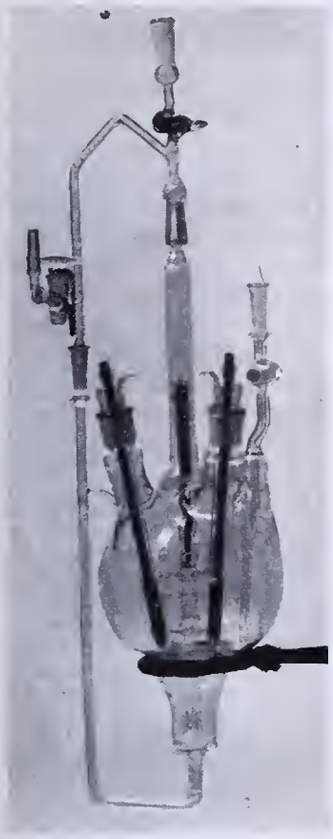

FIGURE 6. DETERIORATION CELL WITH ELECTRICAL MEASURING CELLS AND PROVISION FOR WITHDRAWING SAMPLES

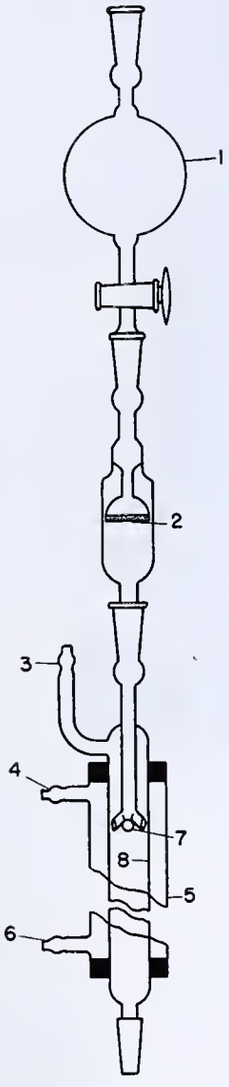

FIGURE 7. OIL-DEGASSING EQUIPMENT

measuring cells (when electrical measurements are desired on the oil sample in the system during the test), are shown in Figures 3, 4, 5, and 6. The cell in Figure 4 is used only when the oxidation kinetics is desired, and the cell in Figure 5 when electrical measurements are to be obtained in addition. A cell of the type shown in Figure 5 is mounted in an oven (Figure 8) with the degassing apparatus assembled for degassing an oil into the cell. The cell of Figure 6 has been used for larger volumes of oil samples, so as to permit withdrawal of samples for chemical tests during the deterioration. This type of cell also has the electrical measuring cells placed in the main body of the sample instead of in connecting tubes as in the cell of Figure 5.

The cell of Figure 5 is shown schematically in Figure 3.

The principal parts are: a chamber, 1, of approximately 150-cc. volume for containing the sample and such contact materials as may be desired; a gas and liquid pump consisting of a plunger with "sealed in" iron core operating in tube 2 and ground-glass ball valves, 3 and 4, with ground seats; tubes 9 and 10 with ground joints 11 and 12 for containing the electrical measuring cells; tube 2 with ground joint 16 which is used for admission of the oil sample into the system and for connection with the automatic pressure regulating the oxygen feed system; support 13 serving as a stop for the reciprocating plunger operating in tube 2; and baffle 14 to prevent the contact materials included with the oil sample, such as copper and paper, from entering tube 7 and fouling the operation of the pump.

The method of operation is as follows: The plunger is given a reciprocating vertical motion by means of a solenoid mounted concentric with tube 2 as shown in Figure 8. The plunger displacement on the upward stroke opens valve 3 and closes valve 4, thus drawing gas from above the sample in chamber 1 through tubes 5 and 6 and then up through valve 3 and tube 7, and dispersing it through the sample in chamber 1. The plunger displacement on the downward stroke opens valve 4 and closes valve 3, thus causing oil to flow through tube 8, upward through valve 4, and then successively through tubes 9 and 10 containing the electrical measuring cells, and into the sample chamber, 1.

Experience with a reciprocating glass plunger operating in a glass tube has shown that excessive grinding of the glass surfaces will take place even when the contact surfaces are under oil. This is prevented in these cells by separating the normal glass contact surfaces by platinum wire fastened to the plunger. This is shown at the top of the plunger in Figure 5. Also the plunger seat (13, Figure 3) is provided with a platinum wire ring to prevent glass-to-glass contact at the bottom of the plunger stroke.

The plungers in these cells have been operated at rates of 10 to 30 strokes per minute, giving a pumping rate for both liquid and gas of the order of 20 to 60 cc. per minute. While these values can be easily increased or decreased, the rates given have been effective.

In the cell shown in Figures 3 and 5, the contact materials such as paper and copper are introduced into the cell through tubes 2 and 5 with the plunger removed from the cell. The paper is in the form of disks, approximately 0.5 cm. in diameter, and the copper in the form of spirals 0.5 cm. in diameter and 2 to 3 cm. in length, using No. 36 copper wire.

The cell of Figure 4 has a ground flange and cover plate which permits use of paper disks approximately the diameter of the cell chamber. These disks have holes in them to permit effective contact of the oil with the paper and adequate gas and liquid circulation. This method permits a maximum amount of paper for a given volume of oil. The ground cover plate is sealed to the flange by water glass applied between the ground surfaces. In the cell in Figure 6 the added materials are put into the cell through one of the ground joints supporting an electrical cell. In this system an oil sample may be removed from the cell through the capillary tubing, stopcock, and ground joint shown, into a sampling tube mounted on the ground joint. The oil sample is forced into the tube by raising the gas pressure within the cell.

The cell of either Figure 3 or 6 is designed to permit electrical measurements to be made on the electrical cells with the system under a vacuum before or after the oil is degassed into the deterioration cell. Following degassing into the system, the oil may be brought to the level of the outlet tube from tubes 9 and 10 containing the electrical cells, by operation of the pump. After electrical measurements are made the system is brought up to 760-mm. pressure with the gas used. Connecting tube 15 in Figure 3 equalizes the pressure above the oil in tube 9 with that of the chamber containing the oil sample and thereby prevents the oil from filling tube 9 after gas is admitted to the system.

Degassing Equipment

At the start of a test, the oil is admitted into the deterioration cell through the degassing equipment. The purpose of degassing

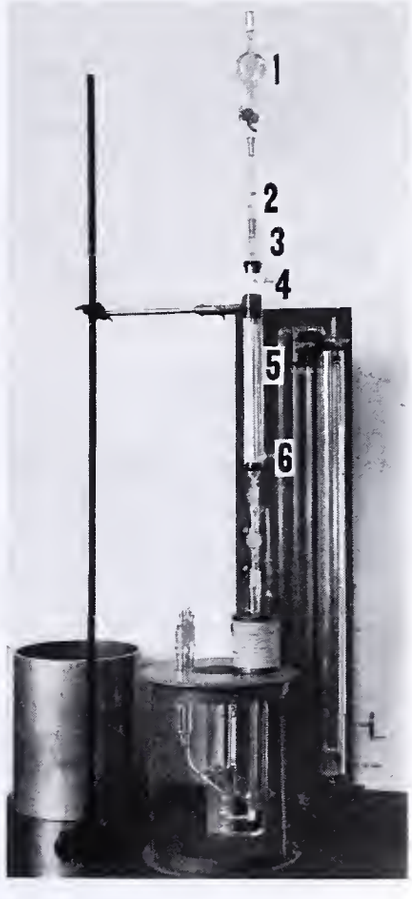

FIGURE 8. DEGASSING EQUIPMENT AND DETERIORATION CELL

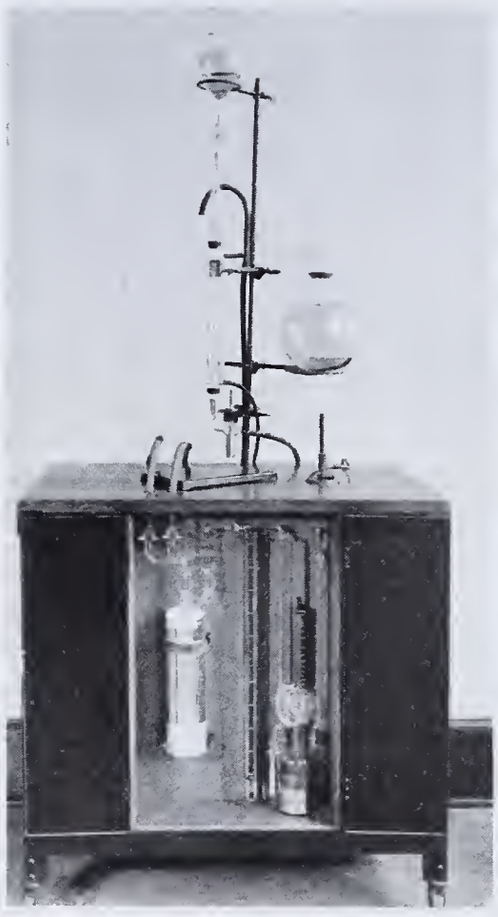

FIGURE 9. DEGASSING EQUIPMENT AND ELECTRICAL MEASURING CELL

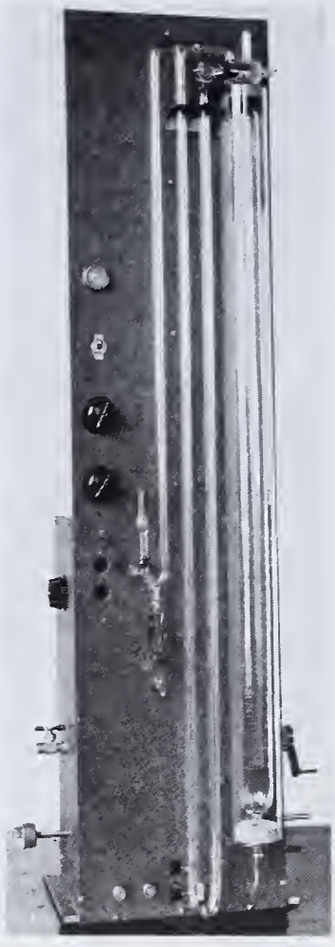

FIGURE 10. AUTOMATIC OXYGEN FEED AND PRESSURE-MAINTAINING APPARATUS

Automatic Gas Feed and Pressure-Maintaining Apparatus

The maintenance of uniform conditions during the oxidation and the accurate determination of electrical and chemical data have required the use of an automatic system for feeding gas (oxygen or nitrogen) to the deterioration cell as reaction with the oil occurs. This system is shown in assembled form in Figure 10.

The component parts are: a 500-cc. water-jacketed buret for containing the oxygen or other gas, and using butyl phthalate as the displacement medium; a hydron bellows (containing butyl phthalate) shown in Figure 11 connected to the 500-cc. buret; a mercury manometer with two platinum contacts, one normally immersed in the mercury and the other making contact with the mercury at a system pressure of 760 mm.; and an automatic displacement mechanism shown in part in Figure 11 for controlling the volumetric displacement of the system to maintain a pressure of 760 mm. in the deterioration cell.

The operation of the system is as follows: When the system pressure decreases below 760 mm., a circuit is made between the two contacts in the manometer. Closing of this circuit in conjunction with a vacuum tube control circuit and relay energizes the solenoid shown in Figure 11. This solenoid when energized draws a wheel (with engaging pin) which is mounted on a steel tube and is normally held in a disengaged position by a spring, into contact with the star wheel shown in Figure 11. The wheel and tube operate on a continuously rotating shaft at slow speed and are driven from the shaft through a clutch. The star wheel

is (*a*) to remove dissolved gas and other volatile products in the oil sample so as to standardize initial conditions, (*b*) to filter the sample to remove any foreign particles, and (*c*) to permit a low pressure to be maintained in the deterioration cell during the admission of the oil, thereby thoroughly impregnating the paper in the cell.

The degassing equipment is shown schematically in Figure 7 and assembled in position for degassing an oil sample into a deterioration cell in Figure 8.

The principal parts of this equipment are: a sampling flask, 1, with male and female ground joints, and a stopcock for controlling the rate at which the oil is admitted to the system at a pressure of approximately 50 microns; a sintered-glass disk, 2, mounted between a male and female ground joint for filtering the oil; an assembly for placing the oil in the form of a thin film and for heating this oil film to approximately 95° C. This assembly consists of nozzles, 7, for spraying the oil onto the interior surface of tube 8, and a jacket formed by tube 5 which permits tube 8 to be heated by steam applied through inlet 4 and drained out at 6. The connection of vacuum pump is made through outlet 3, this arrangement preventing the pump from being fouled by oil.

The oil sample is not heated before admission of the oil to a reduced pressure, and the method of heating the oil at reduced pressure prevents heating in excess of 100° C.

The degassing equipment is also shown in Figure 9 in position for degassing an oil directly into an electric measuring cell (of the type previously described) for electrical measurements under vacuum. This procedure may be used for making precise electrical measurements on the oil sample under carefully controlled conditions.

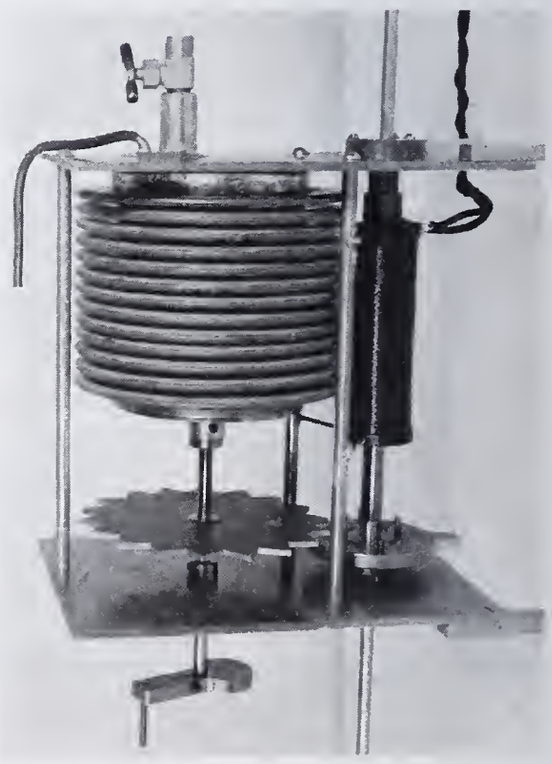

FIGURE 11. MECHANISM WITH HYDRON BELLOWS FOR VOLUMETRIC DISPLACEMENT

is mounted on a threaded shaft which when rotated changes the displacement of the hydron bellows and accordingly the volume of butyl phthalate in the buret.

The control circuit is designed to give a time delay to prevent "chattering" of the mechanism due to system pressure changes produced by the solenoid pump. One notch on the star wheel corresponds to a change in the volumetric displacement in the system of 0.2 cc. The system is provided with manual control of the buret volume by the crank shown in Figures 10 and 11, and provides outlets and controls for oven heating units, a thermoregulator, cooling water for the coils, and control for the solenoid operating the pump. The system as designed thus permits constant pressure conditions to be maintained in the deterioration system, and the rate at which oxygen is consumed and total absorption as a function of time to be determined from buret readings.

Discussion

The apparatus described has been used for studying mineral oil deterioration under a variety of conditions, such as continuous oxidation at 760-mm. pressure, and limited oxidation with only a definite amount of oxygen admitted to the system, the system pressure being maintained with nitrogen (*2*). The equipment described does not include provision for removal of the volatile oxidation products, although this may be effectively accomplished by connecting an absorption train in tube 6 (Figure 3), thereby pumping the gas continuously through the absorption train. An electrical measuring cell, including an absorbent paper, may be placed in the gas line, tube 6, thereby permitting electrical measurements of the volatile products. Experience has indicated that the electrical measurements are of value not only for the specific application of mineral oils for electrical use but also for fundamental information concerning the mechanism of the deterioration.

Acknowledgment

The apparatus described in this paper has been developed in a program of research on mineral oil deterioration which until September 1, 1939, was carried on under the sponsorship of the Utilities Coordinated Research, Inc., Association of Edison Illuminating Companies. Since that date it has been carried on in cooperation with a committee on insulating oils and cable saturants, Herman Halperin, chairman, representing the sponsors, The Engineering Foundation, and the American Institute of Electrical Engineers. Funds for this work are being contributed by a group of electric power companies, a group of oil companies, and a group of electrical manufacturing companies in addition to the sponsors.

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A New Laboratory Fractionating Column Head

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IN CONSTRUCTING and using a laboratory fractionating column for analytical purposes, a good column head is just as important as the column itself, if not more so. A perusal of the brief reviews on laboratory fractionation (*2*, *4*, *7*) reveals the large number of types of columns and heads in use. However, most of them are either very elaborate to construct or very expensive to purchase.

The partial condensation type head of the well-known Peters column (*5*) is probably the simplest form with which the reflux ratio can be varied at will, but with it the amount of reflux cannot be readily ascertained. The column of Cooper and Fasce (*1*) possesses the advantage that the amount of reflux can be determined by counting the number of liquid drops going back to the column. Its drawback is that, as the area of the cooling surface of the partial condenser is fixed, the reflux ratio can be varied only by varying the rate of flow of the cooling medium. With liquids boiling above 100° C. the performance was found by the present author to be unsatisfactory. Furthermore, the thermometer reading of their column tends to be low at slow rate of distillation. The head designed by Simons (*6*) has proved very successful (*3*), but its construction is rather elaborate.

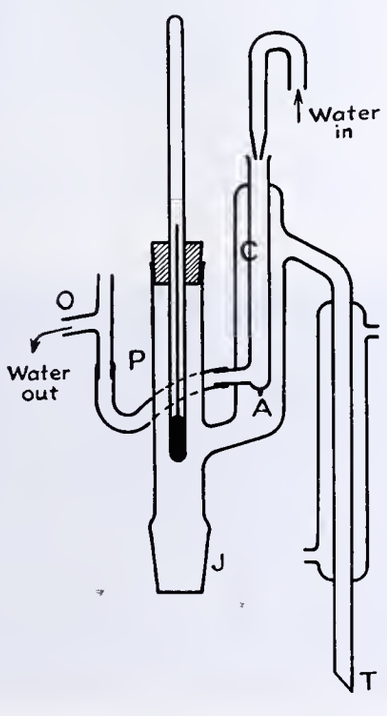

Figure 1

A relatively simple column head has recently been built by the author and is shown in Figure 1. Its performance was found very satisfactory in actual use.

It is a combination of the type of Peters and Baker and of Cooper and Fasce. The level of the cooling water in the partial condenser, *C*, can be adjusted by raising or lowering the outlet tube, *O*, which is not in the same plane with the thermometer pocket, *P*. The reflux ratio can be estimated by counting the liquid drops at both tips *A* and *T*. It must be remembered that the drops at the two tips may not be of the same size and their relative value, if wanted, must be calibrated. The ground joint, *J*, enables the head to be used with different columns.

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Spectrochemical Analysis of Trace Elements in Fertilizers

Boron, Manganese, and Copper

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AS RESEARCH on the nutrition of plants and animals continues, the importance of the presence or absence in their food of the merest traces of certain elements has been more and more emphasized. Thus, for example, it has been shown that the presence of a few parts per million of boron in the soil will prevent the internal cork of apples or the heart and dry rot of sugar beets, but one-half part per million in irrigation water was sufficient to damage some citrus crops in California; application of extremely small quantities of manganese will cure chlorosis of tomatoes or Pahala blight of sugar cane; while copper in only one-sixteenth to one-eighth part per million will prevent the permanent wilt of tobacco, amounts much in excess result in a stunting of plant growth. McMurtrey and Robinson (*4*) have compiled "A Reference List of the Secondary Elements and Their Relation to Plant Development", in which they summarize the known effect of thirty elements on the growth of plant crops. Daniel (*1*) has similarly summarized the role of thirty-three trace elements in animal nutrition.

With the importance of these secondary elements in the growth of plants and nutrition of animals established, knowledge as to their presence and concentration in available mixed fertilizers, where they are possible important sources, seems desirable. Fertilizer companies customarily guarantee only definite percentages of the primary plant nutrients, nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O). The total content of these plant nutrients in the average mixed fertilizer now amounts to about 19 per cent. The question of what constitutes the remaining 81 per cent was answered in this laboratory by Lundstrom and Mehring (*3*) who determined the complete composition of 44 mixed fertilizers chosen on a basis of relative consumption of each grade. The chemical methods therein involved for the determination of important secondary elements, whose concentrations range from only 0.0005 to 0.1 per cent, are both complicated and time-consuming.

It is to this type of problem that spectrochemical analysis is particularly adapted. The spectrograph has the advantages of being direct, thereby avoiding error due to chemical manipulations involved in separations of small quantities; of being rapid; and of achieving, when concentrations are particularly low, accuracy comparable to chemical methods. Accordingly, a study of the application of spectrochemical methods to the analysis of mixed fertilizers has been made (*5*) and a procedure devised which will permit the simultaneous determination of three important secondary elements, boron, manganese, and copper. This paper describes the application of this method to the spectrochemical analysis of the 44 representative mixed fertilizer samples collected and analyzed by Lundstrom and Mehring and compares the results and the time required with the chemical methods.

Apparatus and Method

GENERAL METHOD OF PROCEDURE. As the concentrations of the secondary elements in the mixed fertilizer samples varied over appreciable ranges, the step-sector method of Scheibe (*7*, *8*) was selected. A Hilger seven-step sector with a step ratio of 2 was used when photographing each sample, thus permitting plate calibration and wide selection of the intensity best suited for the photometric measurement of any line. While this procedure limits to seven the number of samples which can be photographed on a single plate, it prevents any possibility of a selected line in the spectrum of any sample being too light or too dark to permit an intensity measurement which will fall within the straight-line portion

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FIGURE 1. SPECTROGRAMS OF MIXED FERTILIZER SAMPLES

of the calibration curve. Thus, by avoiding the necessity of rephotographing at another intensity, it undoubtedly effects a saving in both time and expense when a wide range of concentrations is encountered.

The extreme difficulty of strict control of arcing conditions with the heterogeneous mixed fertilizer samples made advisable adoption of the internal standard method of Gerlach (*2*), where small variations are of no consequence. As it is believed that advantages of the spectrochemical method in both speed and accuracy lie in the directness of the method, a procedure was sought which would necessitate no chemical manipulations. Therefore no attempts were made to dissolve the difficultly soluble fertilizer samples nor to concentrate the small amounts of the elements to be determined by any extraction methods. This necessitated abandonment of the alternating current high-voltage arc, in spite of its other advantages, since studies of this method (*5*) have led to the conclusion that its applicability is limited to analysis of solutions or of powdered materials which are very homogeneous. Hence the low-voltage direct current arc, which permits complete volatilization of a more representative sample, was selected. Figure 1, a reproduction of an entire plate, shows the spectra of seven typical fertilizer samples thus photographed.

Apparatus. The spectrograph is the large Hilger Littrow quartz instrument (No. E-492). The arc stand, patterned after the National Bureau of Standards design, with modifications to provide water cooling of the electrode holders and accurate setting of the electrode separation by a screw motion rather than the rack and pinion, was built to fit the optical bench. The electrode holders are well insulated with soapstone to permit adjustment of electrode position during exposure. A constant electrode separation is maintained by aid of a tenfold enlarged image of the source projected, by means of an appropriately placed lens, between marks on the nearby wall. The seven-step sector is placed about 2 mm. in front of the slit and a lens in front of the sector imaging the source upon the collimator lens provides uniform intensity along the slit height. A variable sector, to control the over-all intensity, is placed in front of this lens. The arc is maintained by means of a 250-line voltage at currents which can be varied by means of a water-cooled variable rheostat.

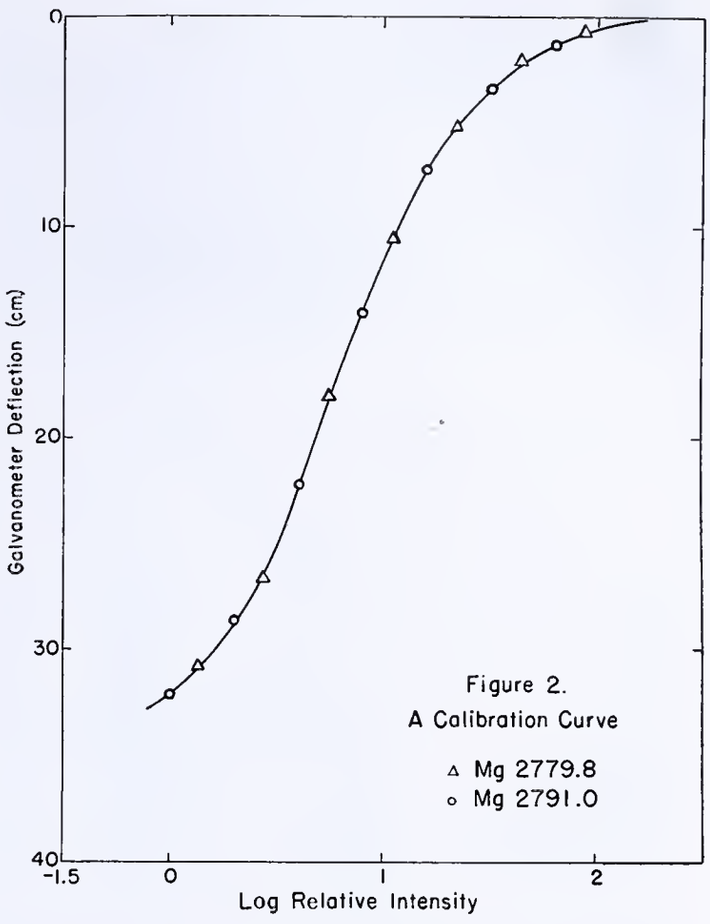
The plates are measured in a Bausch & Lomb nonrecording densitometer. By means of an iris diaphragm described by Scribner (*9*) the full-scale deflection is held constant, so that galvanometer readings can be used directly for plate calibration without calculating opacities or densities. Another useful addition to the densitometer is a slow-motion screw, by means of which the galvanometer reading for various lines can be more conveniently obtained.

Experimental Procedure. The conditions chosen for the determination of boron, copper, and manganese in mixed fertilizers after detailed preliminary study were: a current of 15 amperes, at 250-line voltage, an electrode separation of 4 mm., and a slit 7 mm. high and 0.06 mm. wide. The top electrodes were pointed graphite rods and the lower electrodes were made with a flat-bottomed cup 6 mm. deep, 4.3 mm. in diameter, and with a wall 0.5 mm. thick, shaped by a tool patterned after Myers and Brunstetter (*6*) which cuts the outside and inside of the rod at the same time.

A 10-mg. sample was mixed with twice its weight of pure graphite, as preliminary study has shown that with such a mixture a steadier arc could be maintained. The mixture was placed in the graphite cup, which was made the anode. The lines selected for the analysis were boron 2497.7 Å., manganese 2605.7 Å., and copper 3247.5 Å., and the instrument was adjusted for the region 2450 to 3500 Å. On the basis of studies made on the intensities of these lines, the variable sector was adjusted to eliminate one half of the emitted light. Moving plate pictures show that a 2.25-minute exposure time was required to volatilize the sample completely.

As the complete composition of the fertilizer samples was known, a material to serve as a base for the spectrochemical analysis was prepared to correspond to the average amount of each of the inorganic constituents and 0.05 per cent beryllium was added as a reference element. All chemicals were checked spectrochemically for freedom from boron, manganese, copper, and beryllium impurities.

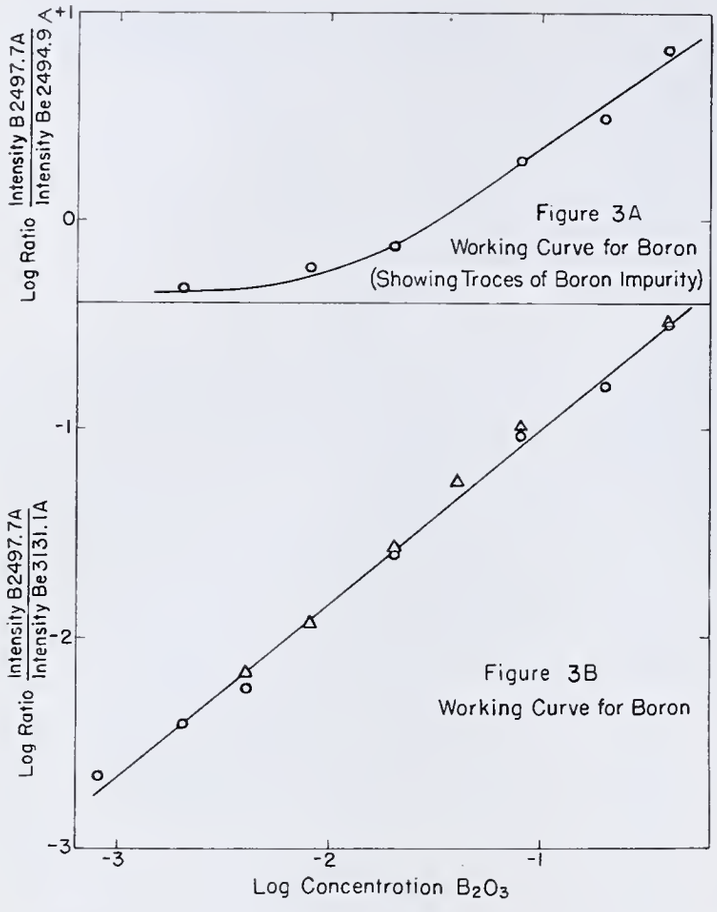
Theoretically an element to serve as an internal standard should meet the qualifications (1) that it possess lines in the spectral region close to the lines selected for the analyses, (2) that spectral lines of the reference element do not interfere with the lines selected for analyses, (3) that the lines of the reference element and the lines selected for the analyses represent similar energy conditions in the arc, and (4) that the reference element volatilize at approximately the same rate as the elements to be determined. A practical criterion of the suitability of an element as an internal standard is simply that it give constant intensity ratios with the lines selected for the analyses. The selection of an element to be added as an internal standard to a substance with a spectrum as complex as that produced by a mixed fertilizer, which will meet all these requirements, is difficult. Beryllium, with lines at 2494, 2650, 3130, and 3321 Å., conforms to the first two conditions only. However, when constant ratios with the selected boron, manganese, and copper lines were obtained with use of the beryllium line at 3130 Å., this standard was selected. This use of a somewhat slowly volatilizing element as an internal standard made complete volatilization of the sample particularly important; otherwise low intensity ratios and consequent high values of the concentrations will result.



Each plate was calibrated by measuring the galvanometer deflection of the seven steps of two lines close together in the spectrum. The logarithm of the relative intensities of the seven steps of each of these lines is plotted against the galvanometer deflections. Figure 2 shows a typical calibration curve so obtained.

Standards were prepared by adding boron, manganese, and copper in amounts varying from 0.0004 to 0.4 per cent to 1-gram samples of the base. Evaporation in a desiccator at rather high vacuum over phosphorus pentoxide was found to be the most satisfactory method of preparing these standards. A solution containing appropriate amounts of the boron, manganese, and copper as well as the beryllium internal standard was added to the 1-gram portion of the base. The solution penetrates the entire sample and, when evaporated in this manner, the sample does not adhere to the evaporating dish as invariably happens when other methods of evaporating—i. e., over a hot plate or steam bath—are used.

The working curves were obtained by plotting, after correction for step and background had been made, the logarithm of the ratio of the relative intensities as obtained from the densitometer readings and the calibration curves, against logarithm of the known concentrations. A background correction for any line is obtained by measuring the blackness on each side of the line and converting the average of these read-



ings to intensity by use of the calibration curve. This intensity value is then subtracted from the measured intensity of the line. Working curves for boron as first obtained (Figure 3-A) showed departure from proportionality at the lower concentrations, indicating the presence of small additional amounts of the element. Further study showed that the boron was present in the graphite rods used, and when the purest grade of carbon was procured, the straight-line working curve (Figure 3-B) was obtained. Similar experience was encountered in the preparation of the working curves for copper and manganese. Only the purest grade rods are suitable for this work. Two sets of standards were prepared and separately photographed. The first set, represented by triangular symbols in Figure 3-B, established the working curves and the second set, represented by circular symbols, served as a check on the experimental accuracy in obtaining the working curves, and as a test of the accuracy of the procedure.

Eastman "Process" plates were selected on the basis of previous tests made in this laboratory (*5*). Eastman developer D-76-c, prepared as instructed, then diluted with 2 parts of developer to one part of water, was used as this gave a satisfactory value of gamma, although studies indicate that the choice of developer was not an important factor (*5*). The plates were constantly brushed while being developed for 5.5 minutes at 18° C.

Results and Discussion

Precision. Table I lists the results obtained from duplicate analyses of the 44 fertilizer samples for boron, manganese, and copper. The average per cent deviations of the du-

Table I. Comparison of Spectrochemical and Chemical Results

Fertilizer Grade[a]	Source	Year Sampled	Boron			Manganese			Copper		
			Spectrochemical[b]	Chemical[c]	Ratio, chemical to spectrochemical	Spectrochemical[b]	Chemical[c]	Ratio, chemical to spectrochemical	Spectrochemical[b]	Chemical[c]	Ratio, chemical to spectrochemical
			Av. %	%		*Av.* %	%		*Av.* %	%	
1*-9-4	N. Y.	1929	0.0070	0.0056	0.80	0.027	0.011	0.41	0.0107	0.012	1.12
2*-8-10	N. Y.	1929	0.00475	0.0049	1.03	0.016	0.0099	0.62	0.015	0.014	0.93
2-12-6	Ohio	1929	0.00435	0.0097	2.23	0.0745	0.0809	1.09	0.0175	0.0040	2.29
7-5-4	Md.	1926	0.0097	0.017	1.75	0.00745	0.0050	0.67	0.0077	0.0024	0.31
4*-8-4	Va.	1930	0.0135	0.010	0.74	0.0135	0.0064	0.47	0.00695	0.0080	1.15
3-8-3	Va.	1930	0.0735	0.0042	0.57	0.013	0.0092	0.70	0.0028	0.0072	2.57
4*-8-4	N. C.	1925	0.00585	0.0028	0.48	0.0085	0.0064	0.75	0.0051	0.0064	1.25
4*-8-4	Va.	1926	0.0335	0.030	0.90	0.0115	0.0057	0.50	0.0010	0.0032	3.20
3-8-5	Md.	1930	0.0069	0.0028	0.41	0.0085	0.0064	0.75	0.0018	0.0032	1.78
4*-12-4	Va.	1930	0.01035	0.0097	0.94	0.016	0.011	0.69	0.0061	0.0095	1.56
5*-8-5	Penna.	1929	0.011	0.010	0.91	0.062	0.0035	0.56	0.00088	0.0032	3.64
5*-8-7	Va.	1930	0.013	0.013	1.00	0.0125	0.0071	0.57	0.00375	0.0072	1.92
7*-6-5	Va.	1930	0.0145	0.0056	0.39	0.011	0.0071	0.65	0.00445	0.0032	0.72
2-9-5	Md.	1935	0.017	0.0049	2.88	0.0185	0.0092	0.50	0.0022	0.0048	2.18
2-12-2	Ind.	1935	0.0070	0.0042	0.60	0.014	0.053	3.79	0.0036	0.0024	0.67
2-12-4	Md.	1935	0.00070	0.0035	5.00	0.098	0.011	0.11	0.00275	0.0040	1.45
3*-9-3	Ga.	1935	0.00625	0.0056	0.90	0.0205	0.018	0.88	0.0019	0.0024	1.26
4*-8-4	Ga.	1935	0.0084	0.0063	0.75	0.0245	0.0071	0.29	0.00195	0.0072	3.69
3-8-6	N. C.	1935	0.0042	0.0056	1.33	0.016	0.0035	0.22	0.00145	0.0008	0.55
4*-8-6	S. C.	1935	0.00545	0.0049	0.90	0.011	0.053	4.82	0.0013	0.0032	2.46
4-8-4	La.	1935	0.0039	0.0049	1.26	0.0135	0.0057	0.42	0.000885	0.0008	0.90
4-8-5	Md.	1935	0.0045	0.0042	0.93	0.0195	0.011	0.56	0.00275	0.0032	1.16
4-8-7	N. Y.	1935	0.019	0.013	0.68	0.038	0.028	0.74	0.0020	0.0064	3.20
4-10-7	Va.	1935	0.0040	0.0049	1.23	0.013	0.0085	0.65	0.0018	0.0008	0.44
4-12-4	Ark.	1935	0.00145	0.0097	0.67	0.0695	0.057	0.82	0.00515	0.0024	0.47
6-6-5	Va.	1935	0.015	0.017	1.14	0.0205	0.011	0.54	0.0035	0.0095	2.71
6*-8-6	Mass.	1935	0.0015	0.0049	3.26	0.017	0.011	0.65	0.00655	0.0008	0.12
3-21-6	Ohio	1929	0.01035	0.014	1.35	0.0465	0.048	1.04	0.0018	0.0048	2.67
4-16-4	N. Y.	1935	0.0023	0.0042	1.82	0.018	0.0078	0.44	0.00235	0.0040	1.70
4*-16-10	Penna.	1930	0.014	0.014	1.00	0.0145	0.011	0.71	0.00165	0.0032	1.94
4-24-12	Ind.	1934	0.0135	0.024	1.78	0.015	0.040	2.67	0.0016	0.0032	2.00
8-12-20	Maine	1935	0.021	0.015	0.71	0.038	0.0014	0.04	0.0012	0.0008	0.67
8*-16-8	Ga.	1927	0.042	0.033	0.78	0.0135	0.0028	0.21	0.056	0.051	0.91
8-16-12	Minn.	1933	0.00175	0.0056	3.20	0.0038	0.0014	0.37	0.0013	0.0064	4.92
8*-16-16	Mass.	1935	0.012	0.021	1.75	0.00815	0.0014	0.17	0.00098	0.0024	2.45
9*-27-9	Va.	1928	0.00375	0.0049	1.31	0.00525	0.0014	0.27	0.0026	0.037	1.42
12-24-12	Germany	1930	0.0025	0.0056	2.24	0.017	0.012	0.71	0.0019	0.0024	1.26
10-30-10	N. J.	1929	0.0087	0.0084	0.97	0.012	0.013	1.08	0.00145	0.0008	0.55
17-34-17	Ala.	1932	0.00047	0.0035	7.45	0.00245	0.0014	0.57	0.00295	0.0024	0.80
0-10-4	Tenn.	1935	0.00735	0.0035	0.48	0.125	0.059	0.47	0.0114	0.0032	0.28
0-10-10	N. Y.	1929	0.00805	0.0049	0.61	0.019	0.0021	0.11	0.00695	0.0072	1.04
0-12-5	Md.	1935	0.0052	0.0021	0.40	0.023	0.0050	0.22	0.0033	0.0032	0.97
0-12-12	Ind.	1934	0.0045	0.021	4.67	0.025	0.012	0.48	0.0021	0.0024	1.14
0-14-6	Ohio	1926	0.022	0.0077	0.35	0.1025	0.056	0.55	0.0029	0.0008	0.28

[a] Grade formulas stand for percentages of N, P_2O_5, and K_2O in this order, except that numbers indicated by an asterisk stand for percentages of NH_3.
[b] Values are expressed as element in accordance with recommendation adopted by Association of Official Agricultural Chemists.
[c] Chemical results from Lundstrom and Mehring are converted to per cent of element to facilitate comparisons.

TABLE II. PRECISION OF SPECTROCHEMICAL ANALYSIS

Trial	Boron Concn.	Boron Deviation from mean	Manganese Concn.	Manganese Deviation from mean	Copper Concn.	Copper Deviation from mean
	%	%	%	%	%	%
1	0.0083	0.0013	0.023	0.0035	0.0095	0.0012
2	0.0068	0.0002	0.028	0.0014	0.013	0.0020
3	0.0070	0.0000	0.028	0.0014	0.011	0.0004
4	0.0070	0.0000	0.031	0.0035	0.010	0.0004
5	0.0070	0.0000	0.023	0.0035	0.0095	0.0012
6	0.0068	0.0002	0.023	0.0035	0.0095	0.0012
7	0.0062	0.0008	0.028	0.0014	0.013	0.0020
8	0.0068	0.0002	0.031	0.0035	0.010	0.0004
Av.	0.0070	0.00034	0.027	0.0027	0.0107	0.0011

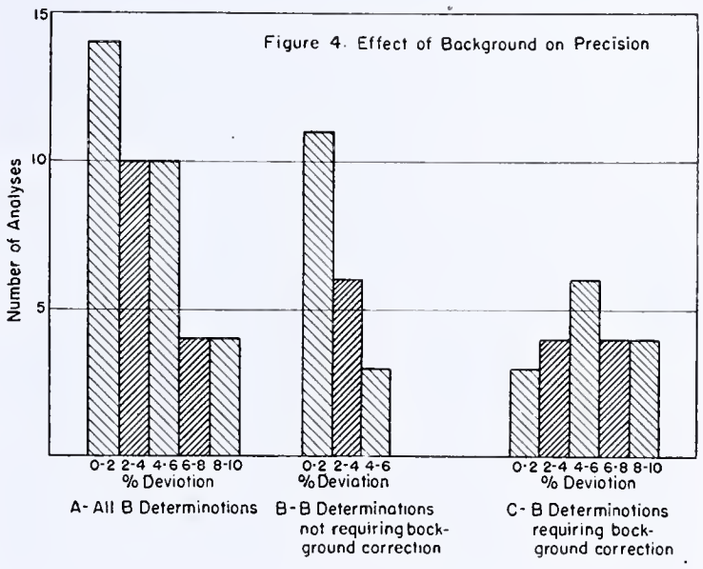
plicate spectrographic determinations from their means are B ±3.58, Mn ±3.61, and Cu ±4.24. The deviations from the average value of the duplicate determinations never exceed ±10 per cent and range from a minimum of 0.0 to maxima of ±9.6 per cent for boron, ±8.3 per cent for manganese, and ±10 per cent for copper.

In Table II are tabulated results obtained from reanalyzing the same fertilizer sample from time to time during the course of this work. The arithmetical mean and the average deviation from the mean are given at the bottom of the columns, under each element. From the data listed the probable error of the mean, $0.6745\sqrt{\frac{\Sigma(d^2)}{n(n-1)}}$, is computed to be 0.00014 for boron, 0.00073 for manganese, and 0.00024 for copper.

The densitometer readings and intensity conversions from the calibration curve for background correction are often difficult to obtain with accuracy. The background intensity is due to compounds radiating band spectra as well as to solid particles emitting continuous light and hence is not uniform in even a given region of a spectrum and its exact value beneath a measured line can only be approximated. The high galvanometer readings of the comparatively weak background, furthermore, had to be read from the calibration curves at low intensity value, often just off the straight-line portion, with consequent further sacrifice of accuracy. It is significant that the average deviation of copper, measured at 3247.5 Å. where appreciable background always necessitated a correction, is greater than that of the boron determinations, measured at 2497.7 Å. where a weaker background made possible many measurements entirely free from correction. The average deviation of manganese is between these two values, but closer to that of boron, just as its measured line, 2605.7 Å., lies in the spectral region.

The boron determinations offer an interesting opportunity to study the effect of background correction upon precision. When the intensity of the boron line is sufficiently strong, measurement can be made free from background, but when the intensity is weak, small background corrections are necessary. Examination shows that of the boron results listed in Table I, twenty were made without background corrections, while in the remainder, corrections were made in either one or both of the duplicate determinations. In Figure 4 the duplicate analyses of boron are charted in five ranges of per cent deviations. Figure 4-A shows the distribution of the total number of determinations throughout these ranges. The importance of background correction is strikingly shown when these duplicate determinations are recharted in two groups, those involving and those not involving background correction, 4-B and 4-C. The average deviation of the determinations made without background correction is ±1.4 per cent, while the determinations requiring background correction show an average deviation of ±5.5 per cent. From these charts the conclusion is gained that the precision of the results could be at least doubled if a more accurate method of measuring background or a method of eliminating it were available.

ACCURACY. With the working curves for each element established from a set of prepared standards, analysis of an additional set to obtain more points on these curves becomes a check upon the accuracy of the entire procedure. The results for boron from two independent sets of standards, shown on the working curve, Figure 3-B, were similar to those obtained for manganese and copper.



Such a check upon the accuracy of the determinations, however, ignores any errors due to differences in the constituents making up the base material and in the fertilizer samples themselves. This source of error can be best avoided, of course, by duplicating as closely as possible the material to be analyzed when preparing the standards, and for this reason the available chemical analyses of the mixed fertilizers were carefully followed in preparing the boron, manganese, and copper standards. However, an additional check on the ac-

TABLE III. ACCURACY OF SPECTROCHEMICAL ANALYSIS

Fertilizer Grade[a]	Source	Year Sampled	Added	Original Analysis	Subsequent Analysis
			%	*Av.* %	%
			Boron		
1*-9-4	N. Y.	1929	0.027	0.0070	0.029
4*-8-4	Va.	1926	0.027	0.034	0.057
4* 8-4	Ga.	1935	0.014	0.0084	0.022
6*-8-6	Mass.	1935	0.014	0.0015	0.016
12-24-12	Germany	1930	0.042	0.0025	0.045
0-14-6	Ohio	1926	0.042	0.022	0.070
			Manganese		
1*-9-4	N. Y.	1929	0.014	0.027	0.040
4*-8-4	Va.	1926	0.014	0.012	0.024
4*-8-4	Ga.	1935	0.0071	0.024	0.026
6*-8-6	Mass.	1935	0.0071	0.017	0.027
12-24-12	Germany	1930	0.021	0.017	0.038
0-14-6	Ohio	1926	0.021	0.102	0.103
			Copper		
1*-9-4	N. Y.	1929	0.016	0.0107	0.028
4*-8-4	Va.	1926	0.016	0.0010	0.013
4*-8-4	Ga.	1935	0.0080	0.0019	0.014
6*-8-6	Mass.	1935	0.0080	0.0065	0.014
12-24-12	Germany	1930	0.024	0.0019	0.021
0-14-6	Ohio	1926	0.024	0.0029	0.024

a Grade formulas stand for percentages of N, P_2O_5, and K_2O in this order; except that numbers indicated by asterisk stand for percentages of NH_3.

curacy which includes this factor was undertaken. To six of the fertilizer samples selected at random, definite amounts of boron, manganese, and copper were added and the samples were reanalyzed. The results and comparisons are shown in Table III.

Comparison with Chemical Results. A comparison of the average of the spectrochemical determinations with the chemical results obtained by Lundstrom and Mehring (*3*) are included in Table I. While in a few cases the ratios of the chemical to the spectrochemical values vary appreciably from unity, in general these ratios are within a twofold variation. There is no significant trend in the comparison of the two methods, although the spectrochemical results for boron and manganese tend to be somewhat higher than the chemical, while the opposite is true of copper. Study to determine a possible cause for the discrepancy between the two methods has not been attempted. When the difficulties encountered in the separation of these small amounts from the complex mixed fertilizers for their chemical determination are considered, a twofold variation is, perhaps, not surprising.

The spectrograph provides a method whereby the usual analyses for the important primary plant nutrients in the fertilizer can be supplemented by a ready procedure for the determination of the secondary elements important to plant nutrition. Once the method described is established as a routine procedure, an individual could maintain an average of complete analysis of two or three photographic plates a day. This would involve the determination of three elements in from 14 to 21 samples, or from 42 to 63 determinations a day. Working curves permitting determination from a few parts per million to a few per cent could be established to correspond to the composition of the average fertilizer with supplemental working curves for the unusual samples—i. e., a fertilizer containing practically no lime. Upon the completion of the usual chemical analyses for the major constituents the spectrochemical analyst would have sufficient information for selecting the most appropriate working curve, and the determination of the concentration of essential secondary elements and the absence (above guaranteed values) of those elements producing toxic effects could rapidly follow.

Summary

Using the low-voltage direct current arc and a method involving a step sector and internal standard, a procedure for the simultaneous determination of three of the more important secondary elements in mixed fertilizers, boron, manganese, and copper, has been found to give satisfactory results with an accuracy of about ±5 per cent.

Study has shown that unavoidable background is the greatest single source of inaccuracy, and that preparation of base material to correspond to the average composition of fertilizers is satisfactory to avoid any error due to the effect of one ion on the excitation of another.

The spectrochemical method can be used to supplement the chemical analysis of fertilizers for primary nutrients by providing a rapid method for guaranteeing the concentration of the essential trace elements.

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Routine Determination of Phosphorus and Sulfur in Coke

Catalytic Nitric-Perchloric Acid Digestion Method

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WET oxidation of coal and coke to determine sulfur has been suggested (*2, 11*). This paper outlines an analogous procedure for phosphorus and presents a modification of the Smith (*11*) procedure for sulfur in coke.

Recent investigations of the structure of coke have shown that nitric acid oxidation converts a large percentage of coke into mellitic and oxalic acids (*3*). As graphitic carbon is easily oxidized by perchloric acid when chromium and manganese are present, no difficulties should be encountered in the rapid oxidation of coke.

In the procedures described below phosphorus is considered present wholly as phosphate, while sulfur may occur as ferrous sulfide, sulfate, free adsorbed sulfur, and sulfur-carbon solid solution (*7, 8*).

The hazards involved are no greater than those encountered in the digestion of rubber or of cast iron. It is best to add nitric acid to coke first, to take care of any volatile matter. Coke and perchloric acid should not be heated unless nitric acid is present.

Reagents Used

Zinc oxide–nitric acid solution, prepared by adding 200 grams of sulfur-free zinc oxide to 1 liter of concentrated nitric acid.

Catalyst. Equal weights of potassium permanganate and potassium dichromate, ground separately, and then mixed. Use about 60 mg. for each determination.

Procedure for Phosphorus

Weigh 1 gram of 60-mesh coke and about 60 mg. of catalyst. Transfer to a tall-form, narrow-mouthed 500-cc. Erlenmeyer flask, and cover with 20 cc. of fuming nitric acid (specific gravity 1.5), 18 cc. of technical phosphorus-free perchloric acid (60 or 70 per cent grade), 1 drop of liquid bromine, and about 1 cc. of hydrofluoric acid. Place on a hot plate and boil gently for 10 minutes, then increase the heat to boil out the nitric acid and oxi-

dize the chromium to chromic acid (203° C.). This should take not less than 45 minutes.

Cool somewhat and add about 25 cc. of water to dissolve the solid matter and 5 cc. of 3 per cent hydrogen peroxide. (The titanium content may be determined at this point, if required, *9*.) Filter, wash with 1 per cent nitric acid, boil out the peroxide, adjust the acidity, and test for phosphorus (*4*). The following technique may be used: To the filtered solution add 6 cc. of nitric acid (specific gravity 1.42), boil out the peroxide, cool to room temperature, and dilute to 75 to 100 cc. Add 10 grams of ammonium nitrate and 50 cc. of molybdate solution (*4*), stir well, and let settle. If the phosphorus content is low, estimate by comparison with a standard steel from which phosphorus has been precipitated at the same temperature as from the coke. Otherwise, filter and determine by the customary alkalimetric method.

If the titanium content is over 0.3 per cent, add 2 cc. of hydrochloric acid (specific gravity 1.2), and allow the precipitate to settle overnight.

Table I. Determination of Phosphorus

Proposed Method	Perchloric Acid Method[a]	Nitric-Hydrofluoric Acid Method[b]
%	%	%
0.012, 0.012	...	0.012
0.012, 0.012	0.008	...
0.024, 0.024	0.020	0.026
0.032[c]	...	0.032
0.040, 0.042[d]	0.040[d]	0.040[d]
0.044	...	0.042

[a] Ash 1 gram, add HF-$HClO_4$, fume, and complete as usual.
[b] Ash 1 gram, add HF-HNO_3, evaporate, take up with HNO_3, and complete as usual.
[c] Manufacturers' result, 0.028 per cent.
[d] Titration by another analyst.

Procedure for Sulfur

Weigh 1 gram of 60-mesh coke and about 60 mg. of catalyst. Transfer to a tall-form narrow-mouthed 500-cc. Erlenmeyer flask, and cover with 10 cc. of the zinc oxide–nitric acid solution. Add 20 cc. of fuming nitric acid (specific gravity 1.5), 20 cc. of C. P. perchloric acid, 1 drop of liquid bromine, and about 1 cc. of hydrofluoric acid. Cover with a watch glass, place on a hot plate, and boil gently. After 10 minutes increase heat, boil out the nitric acid, and oxidize the chromium to chromic acid 203° C. This should take not less than 45 minutes.

Alternate A. Remove watch glass, add 5 to 6 drops of concentrated hydrochloric acid from a dropping bottle, and repeat the additions of hydrochloric acid after the chromium has been reoxidized to chromic acid, but remove the flask from the hot plate to prevent the remaining chromium from being reoxidized also (*6*).

Alternate B. Remove the flask from the hot plate, add 2 cc. of hydrochloric acid, reheat, and then cool somewhat.

Add about 25 cc. of water, heat to boiling, filter, and wash with hot water. Using methyl orange, neutralize the filtrate with ammonium hydroxide, and then make acid with hydrochloric acid, adding 2 cc. excess. Add about 0.25 gram of hydroxylamine hydrochloride to reduce iron and chromic acid, dilute to 200 cc., heat to boiling, and slowly add 10 cc. of a 10 per cent barium chloride solution, while stirring. Continue boiling gently until the solution has cleared and the sulfate has settled. Stir in a little paper pulp. Filter through a No. 42 Whatman paper. Wash with hot barium chloride wash solution (*5*) (1 per cent hydrochloric acid and 0.1 per cent barium chloride). Finally, wash with water, burn, and weigh as usual.

Discussion

The procedure is therefore: (1) oxidizing the carbon rings with fuming nitric acid, (2) dissolving the ash with hydrofluoric acid, (3) consuming the residual carbon with perchloric acid in the presence of the catalyst, and (4) preparing the resulting solution for either phosphorus or sulfur precipitation.

The range of phosphorus in the samples of coke available was from 0.01 to 0.04 per cent, and the range of sulfur was from 0.5 to 1.0 per cent. Sulfur was run by the Eschka method (*1*) and checked against the wet method (Table II). In Alternate B the conditions are those of the final steps in the determination of sulfur in steel (*10*), while in Alternate A most of the chromium could be volatilized without loss of sulfur.

In determining phosphorus it is not necessary to volatilize the chromium or even to reduce from hexavalent to trivalent.

When run side by side, the sulfur digestion is slower than the phosphorus, as only fuming nitric acid is used in the phosphorus determination while in the sulfur determination some nitric acid of 1.42 specific gravity is present. This indicates that the strong nitric acid is important and should not be boiled out too rapidly. Mechanical loss is also at a minimum when digestion is not too rapid.

Omitting hydrofluoric acid with cokes of 10 per cent ash gives low results for phosphorus and sulfur. Bromine is deemed essential with organic compounds containing sulfur, and the zinc compound retards volatilization of sulfuric acid if too much perchloric acid is inadvertently boiled out. The catalyst and the perchloric acid oxidize any remaining carbon, break up the ash, and indicate completeness of digestion.

In Table I results obtained with the proposed method are compared with those employing dry-ashing for the determination of phosphorus.

As compared with Smith's method for sulfur in coke (*11*), this modification probably owes its success to the use of fuming nitric acid, hydrofluoric acid, and bromine. Johnson, who based his sulfur method (*2*) on the permanganate oxidation of coke, used too small a sample (0.2 gram) and too much reagent (5 grams of permanganate, 50 cc. of nitric acid, 60 cc. of perchloric acid, and 50 cc. of hydrochloric acid).

The proposed method gives the same precision of results as the Eschka method (Table II), and is more rapid and easier to handle. Phosphorus and sulfur can be digested and later filtered side by side. Since the analyst need not wait for ashing before proceeding with the phosphorus analysis, both the phosphorus and sulfur determinations may be completed well within 5 to 6 hours.

Table II. Determination of Sulfur

Eschka Method	Alternate A	Alternate B
%	%	%
0.56	..	0.58, 0.58
0.69	0.68	0.68
0.70	..	0.68
0.75	..	0.75, 0.74, 0.74
0.77	..	0.74, 0.73
0.80	..	0.81, 0.80, 0.80
0.81	0.81	0.79
0.86	..	0.89, 0.86
0.88	0.89	0.86
0.96	0.96	0.96
1.03	..	1.05, 1.02
..	1.06	1.08

Summary

An improved procedure for the wet determination of sulfur in coke and an original procedure for phosphorus are given, using the newer theories on the structure of coke as a basis for the method. The digestion acids are nitric and perchloric, not perchloric acid alone.

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Role of Velocity Gradient in Determining the Cuprammonium Fluidity of Cellulose

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IN ATTEMPTING to apply certain routine methods for determining the fluidity of raw cotton cellulose, as a measure of its textile quality, serious difficulties were encountered. These were due to the anomalous behavior of the solutions, which was much more pronounced in the case of native cotton cellulose than in many "finished" cottons, rayons, and other cellulose derivatives.

The literature dealing with the routine measurement of the cuprammonium fluidity or viscosity of cellulose generally recognizes the anomalous nature of the solutions. Thus, Clibbens and Geake (*5*) so outlined their technique that failure to obey the Poiseuille law was made evident, but they did not indicate any alternate procedure in case of deviation. In 1929, the Committee on Viscosity of Cellulose of the AMERICAN CHEMICAL SOCIETY (*1*) recognized the "plastic" behavior of cuprammonium solutions of cellulose but questioned the practical significance of such effects. In 1932 the Fabrics Research Committee (*7*), recognizing the "instrument effect", gave detailed and exacting specifications for the construction of capillary viscometers, hoping in this way to reduce the "plastic" effect to a minimum. Neither the T. A. P. P. I. (*28*) method for determination of cuprammonium fluidity of cellulose, nor the new tentative method adopted by Committee D-13 of the American Society for Testing Materials (*2*) attempts any adjustments for the non-Newtonian flow.

The requirements for treating the viscosity of anomalous solutions have been extensively studied in Europe by a number of investigators. The literature has been adequately summarized for cellulose by Philippoff (*16*). However, while a satisfactory theoretical ground seems to have been laid, the necessary procedures are not readily adaptable for routine applications. Either for this reason or through lack of knowledge of the new developments, little or no attempt seems to have been made to take advantage of them.

It is the purpose of the present paper to describe experiments in which an attempt is made to adapt some of the new techniques for routine or commercial use.

Apparatus and General Procedure

For the fluidity determinations viscometers were employed which were similar to the consistometers described by Herschel and Bulkley (*11*). The parts, general arrangement, and dimensions are shown in Figure 1.

The burets were graduated at 5-ml. intervals and designed to deliver four, or in a few cases, five successive 5-ml. quantities during a complete discharge. A series of capillaries was used, all of which had a length of 2.50 ± 0.02 cm., an outer diameter of 0.7 cm., and an inner radius varying between the limits of 0.0702 and 0.0737 cm. In the final calibration, the capillary radius was determined accurately by means of mercury.

The mean heads, h, of the burets were computed for each 5-ml. portion discharged, by means of Meissner's approximation formula:

$$h = \frac{h_1 - h_2}{\log_e h_1/h_2} \qquad (1)$$

where h_1 and h_2 are the initial and final distances between the level of the column and the lower tip of the capillary. The mean heads were converted into pressures, in grams per square centimeter, by multiplication with the density of the cuprammonium hydroxide solution used—namely, 0.974—no account being taken of the slight increase in density that results from the solute.

In some of the determinations the hydrostatic pressure was reduced or augmented, externally, by means of a by-pass bubbling arrangement in which an escape of air was provided from a comparatively large glass tube (7 mm. in inner diameter) through the desired head of water in a tall glass cylinder. The supply pressure (either positive or negative) was adjusted by means of a stopcock until a slow steady stream of bubbles escaped. The superimposed pressure was measured by means of a water manometer inserted in a 4-liter bottle contained in the supply air line.

The composition of the cuprammonium reagent corresponded to the specifications of the Committee on Viscosity of Cellulose of the AMERICAN CHEMICAL SOCIETY (*1*). It was found to have a fluidity at 25° C. of 82.25 rhes. This composition was used in preference to that of a solution, such as that of T. A. P. P. I. (*28*), containing only 15 grams of copper per liter because, as reported by Conrad (*3*), it dissolved raw cotton cellulose much more readily.

Before solution in the cuprammonium reagent the cotton was purified by extraction for 4 hours with 95 per cent ethyl alcohol in a large Soxhlet apparatus. Ethyl alcohol was found to be a much more inclusive solvent for the heterogeneous waxy constituents than carbon tetrachloride, benzene, or even a 50-50 alcohol-benzene mixture. Also the alcohol removes residual sugars and other substances, not removed by carbon tetrachloride or benzene. No bleaching was employed because of its possible damaging effect on the sample and because of the absence of lignin in cotton fiber. There was no evidence that the fractional per cent of pectic substance remaining on the fiber interfered with the solution of the

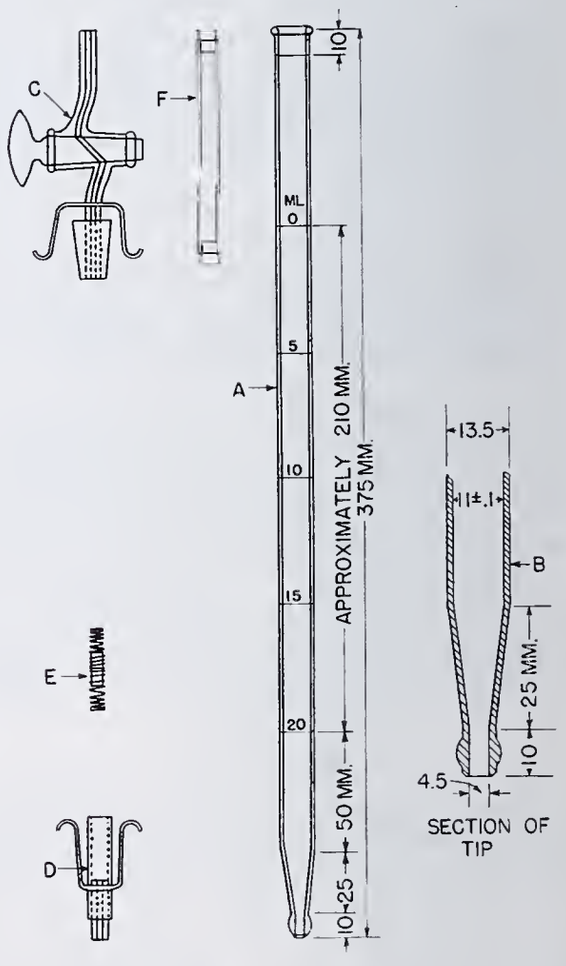

FIGURE 1. PARTS AND DIMENSIONS OF BURET VISCOMETERS

A. Plan of buret, showing position of the six etched rings
B. Enlarged cross section through lower end
C. Stopcock assembly for insertion to ring at top
D. Calibrated capillary and rubber tube assembly
E. Weighted spring
F. Sample transfer tube

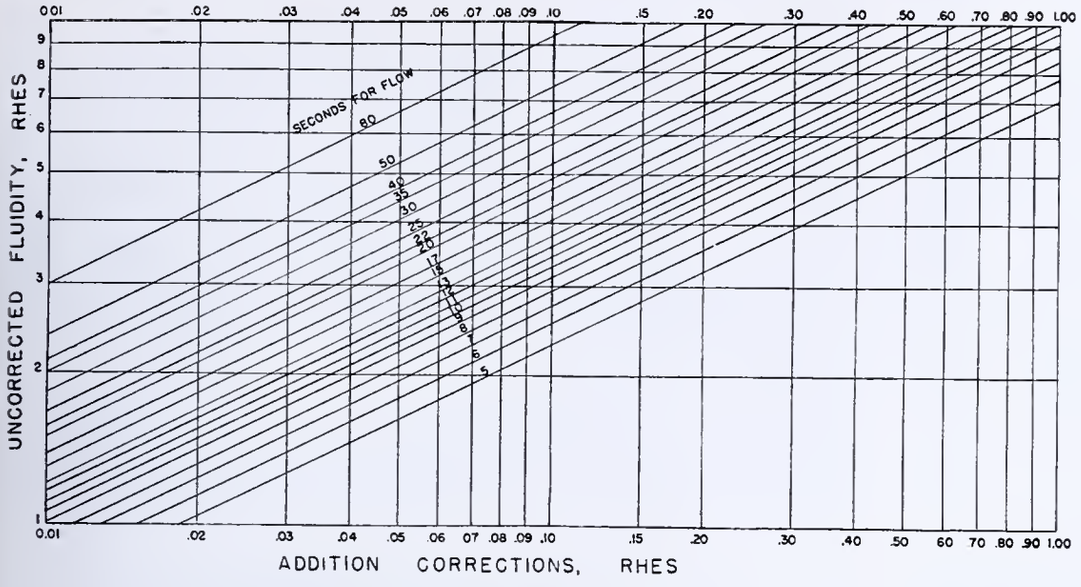

FIGURE 2. ADDITION CORRECTIONS FOR KINETIC ENERGY LOSSES

In relation to fluidity without kinetic energy correction and time of discharge for 5-ml. portions of solutions

cellulose or the results. However, the cellulose content of the sample was determined and used for concentration adjustments.

In preparation for the determination a weighed portion of the sample, sufficient to give an approximately 0.5 per cent solution, was introduced by means of the sample tube (Figure 1, *F*) into the buret, together with a small spring weight (Figure 1, *E*) to promote agitation later. The buret was then flushed out with approximately 3 volumes of purified nitrogen, evacuated to a pressure of about 5 cm. of mercury, and again refilled with pure nitrogen to eliminate oxygen as completely as possible. Without disconnecting, the cuprammonium hydroxide solution was introduced until the buret, which was in an inclining position, was approximately two-thirds full. Care was taken that all of the sample was wet by the reagent, whereupon it was given a vigorous shaking. The buret was then completely filled with the solvent and placed in an enclosed, light-tight, rotating box and allowed to turn end-over-end overnight at the rate of about 2 revolutions per minute. Next morning the burets were placed in a close-fitting glass jacket in a thermostat bath, controlled at 25° ± 0.1° C., and allowed to stand 20 minutes for the attainment of equilibrium temperature. The time required for the discharge of the 5-ml. volumes was then determined with a "split second" stop watch.

The results for 0.5 gram of cellulose per 100 ml. of solution are expressed for textile purposes in terms of fluidity rather than of viscosity, since, as pointed out by Clibbens and Geake (*5*), the relation between fluidity and loss of strength is nearly linear, and the practical range of the absolute fluidity scale is much more convenient for textile celluloses than is that of the viscosity scale.

Kinetic Energy Corrections

Corrections were made in all cases for kinetic energy losses.

This was done by first computing the fluidity without kinetic energy correction and then adding the correction. The corrections were approximate and were taken from a chart such as is shown in Figure 2, based on the evaluation, for convenient values of observed fluidities, F_o, and times of flow, t, of the usual expression for kinetic energy correction:

$$\frac{1}{F_o} - \frac{1}{F_c} = \frac{mdV}{8\pi Lt} \qquad (2)$$

Here, F_c is the corrected fluidity, m is the kinetic energy coefficient, usually taken as 1.12, d is the density of the liquid, V is the volume of liquid discharged in t seconds, and L is the length of the capillary. When the differences between F_c and F_o are plotted in logarithmic coordinates against the uncorrected or observed fluidities for different observed times of flow, a series of straight lines results from which addition corrections can be read off by inspection.

Concentration Corrections

Since the fluidities were expressed in terms of 0.5 per cent solutions, it was necessary to make a second adjustment of all observed fluidity values for small deviations of concentration. This was necessitated by small variations in capacities of the burets as well as variations in cellulose content of samples.

The most convenient method for interpolating fluidity for different concentrations was found to be that based on a formula published by Farrow and Neale (*8*):

$$1 + A/C = B/\log \eta_r \qquad (3)$$

A and B are empirical constants, C is the concentration of the cellulose in grams per 100 ml. of solvent, and η_r is the relative viscosity of the solution. A plot of $1/C$ against $1/\log \eta_r$ should give a straight line. Farrow and Neale confirmed that the linearity of such a plot was approximately realized in practice, and that B had a value of about 11.

The Fabrics Research Committee (*7*) re-examined the concentration viscosity relationship and found rather good linearity of the curves. However, they found a somewhat smaller value for B, this varying between 5 and 10, the more highly viscous samples having the higher values. The writer recomputed some data given by Staudinger and Sorkin (*27*) for gel solutions of nitrocellulose of different degrees of polymerization in butyl acetate and found that here, too, a plot of $1/C$ against $1/\log \eta_r$ gave practically straight lines and a value of B varying between about 4 and 10. In the small region of $1/C$ between 1.5 and 2.5, within which all interpolations would fall, the curves may be taken as linear without appreciable error. For use, an arbitary value of $B = 10$ was adopted and a table of corrections prepared for deviations of concentration from 0.5 per cent.

Demonstration of Anomalous Behavior of Solutions of Raw Cotton Cellulose

The anomalous behavior of the solutions may best be shown by three different methods of treating the data from two solutions of cotton cellulose, and, for comparison, a mineral oil and an aqueous 71 per cent solution of glycerol.

According to the first procedure, the fluidity is computed for each ring interval of the viscometer according to the formula of Clibbens and Geake:

$$F_o = C/t \qquad (4)$$

in which F_o is the observed fluidity in rhes, before correction for kinetic energy, C is the viscometer constant for a particular ring interval and already adjusted for density of the cuprammonium solvent, and t is the time in seconds for discharge between rings.

According to the second procedure the raw data are treated according to Bingham's plasticity method (*4*, p. 323) employing the formula:

$$F_o = \frac{V}{C(P - y)\,t} \qquad (5)$$

In this formula F_o is really Bingham's "mobility" value, although for purposes of comparison it will be referred to as "fluidity", t has the same significance as above, C is a constant having a different numerical value from that of Equation 4, V is the volume in milliliters of solution delivered between two successive rings, P is the total computed pressure causing flow, and y is the "back pressure" or yield value. (There may be some question as to whether the term "mobility" is strictly applicable in the case of solutions of cellulose, since the back pressure, y, is only apparent and unreal. When y is equal to the capillary rise the values calculated by this formula are exactly comparable to those computed with the aid of Equation 4.) The value of y is found by a graphical method as the intercept on the P axis when V/t is plotted

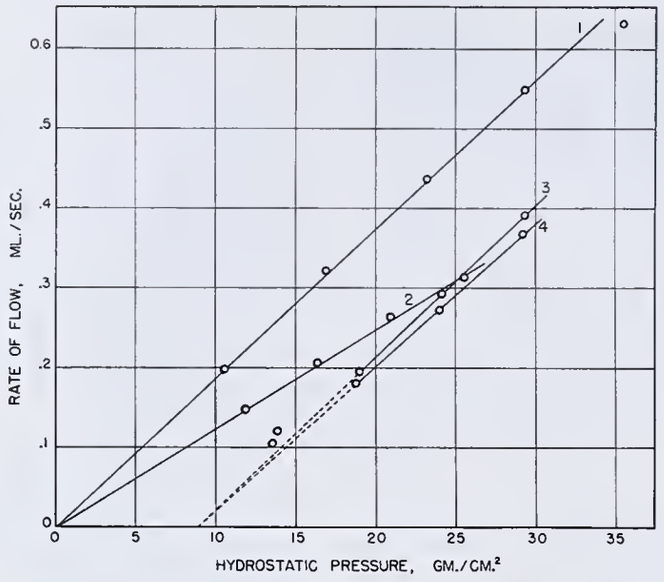

FIGURE 3. RATE OF FLOW-PRESSURE CURVES FOR "TRUE" AND ANOMALOUS LIQUIDS

1. Approximately 71 per cent aqueous glycerol ("true" solution)
2. Mineral oil ("true" liquid)
3. 0.5 per cent cuprammonium solution of cotton cellulose 293 (anomalous solution)
4. 0.5 per cent cuprammonium solution of cotton cellulose 295 (anomalous solution)

against P. Such plots are shown in Figure 3 for a "true" liquid and solution, and two anomalous solutions. Whereas the curves for the "true" liquids when extended pass almost through the origin, the curves for the two cotton solutions (anomalous) when extended intersect the abscissa at nearly 9 grams per square centimeter. [Theoretically, even for liquids that obey the Poiseuille law the curves should pass through a point on the pressure axis slightly to the right of the origin, owing to the influence of surface tension. According to Herschel and Bulkley (*11*) this will be at $P = \frac{4\gamma \cos\theta}{gD}$, where γ is the surface tension of the solution, θ is the angle of contact of the liquid with the surface, g is the gravitational constant, and D is the outer diameter of the capillary. Taking cos θ equal to 0.5 and D as 0.7 cm., the surface tension correction was about 0.20 gram per square centimeter in the viscometers described above.]

The third method of treating the data, the velocity gradient method, will be described subsequently but the data are included here for comparison. The results, corrected for kinetic energy losses and variations of concentrations, are summarized in the last three columns of Table I.

By reference to Table I it will be seen that the fluidity of the mineral oil and the glycerol solution was practically constant, regardless of ring interval (pressure) or method of treating the raw data—i. e., the solutions showed Newtonian flow. However, the two cotton solutions exhibited different fluidities, depending on the method of treatment of the data and, for two of the methods, on the ring interval of the viscometer. The variation with ring interval, depending as it does on applied pressure, is shown especially in the results obtained by the Clibbens and Geake formula, where the total decrease from upper to lower ring interval amounted for the two cotton solutions to about 45 per cent of the average fluidity value.

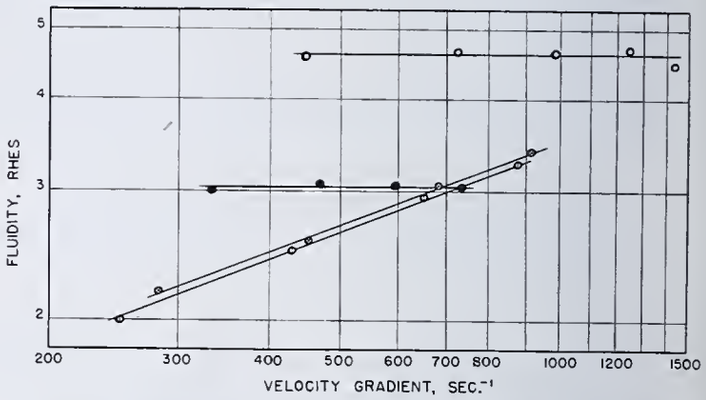

FIGURE 4. RELATION OF FLUIDITY OF "TRUE" AND ANOMALOUS LIQUIDS AND SOLUTIONS TO MEAN VELOCITY GRADIENT

○. Approximately 71 per cent aqueous glycerol ("true" solution)
●. Mineral oil ("true" liquid)
⊗. 0.5 per cent cuprammonium solution of cotton cellulose 293 (anomalous solution)
◐. 0.5 per cent cuprammonium solution of cotton cellulose 295 (anomalous solution)

The fluidities for the two cottons by Bingham's plasticity formula are naturally larger than those by the Clibbens and Geake formula, but rather uniform, except for the lowest ring intervals. Figure 3 shows that the points for the lower pressures on the two cotton curves fall well above the straight-line portion of the curves. In some cases no three of the four sets of observations could legitimately be fitted to a straight line, so that the yield value, y, was uncertain. Superimposition of a small air pressure on the solutions during flow was tried with a view to bringing the points higher and, therefore, more nearly on the straight-line portion of the curve. However, this not only greatly increased the kinetic energy correction, but changed the yield value and, in some cases, resulted in turbulent flow for the highest ring interval.

On the other hand the fluidities of the two cotton solutions by the velocity gradient method, while much lower, are as uniform for the different ring intervals for a given cotton, as are those for the mineral oil and glycerol solution for all ring intervals and methods of treating the data.

TABLE I. FLUIDITIES OF MINERAL OIL, AQUEOUS 71 PER CENT GLYCEROL, AND 0.5 PER CENT COTTON CELLULOSE

Sample and Capillary Radius[a]	Viscometer Ring Interval	Mean Pressure	Mean Rate of Flow	Mean Velocity Gradient	Fluidity by Method of: Clibbens and Geake	Bingham's plasticity[b]	Velocity gradient[c]
	Ml.	*G./sq. cm.*	*Ml./sec.*	*Sec.⁻¹*	*Rhes*	*Rhes*	*Rhes*
Mineral oil,	0–5	25.54	0.315	731	3.04	3.04	3.04
R = 0.0721 cm.	5–10	20.94	0.236	595	3.05	3.05	3.05
	10–15	16.37	0.208	469	3.07	3.07	3.07
	15–20	11.86	0.148	334	3.00	3.00	3.00
				Av. 532	3.04	3.04	3.04
Glycerol	0–5	35.66	0.625	1430	4.47	4.47	4.42
(approx. 71%),	5–10	29.31	0.538	1241	4.69	4.69	4.66
R = 0.0722 cm.	10–15	23.19	0.427	982	4.65	4.65	4.65
	15–20	16.90	0.314	724	4.66	4.66	4.71
	20–25	10.54	0.196	448	4.58	4.58	4.65
				Av. 965	4.61	4.61	4.62
Cotton 293,	0–5	29.36	0.394	913	3.40	4.94	2.71
R = 0.0715 cm.	5–10	24.21	0.294	682	3.06	4.91	2.74
	10–15	19.02	0.195	453	2.57	4.91	2.75
	15–20	13.78	0.121	282	2.19	6.38	2.72
				Av. 566	2.80	5.28	2.73
Cotton 295,	0–5	29.25	0.368	873	3.28	4.74	2.65
R = 0.0710 cm.	5–10	24.01	0.273	649	2.95	4.70	2.67
	10–15	18.77	0.181	430	2.49	4.72	2.64
	15–20	13.53	0.105	250	2.00	5.66	2.68
				Av. 550	2.68	4.96	2.66

[a] Length of capillaries in all cases 2.50 cm.
[b] Strictly "mobility" although called "fluidity" for comparison.
[c] Interpolated to velocity gradient of 500 sec.⁻¹

Elimination of "Instrument" Errors

In the case of anomalous solutions the disturbances may be divided, for convenience of discussion, into two categories. One has to do with the instrument effect—i. e., the difference in fluidity or viscosity of an anomalous

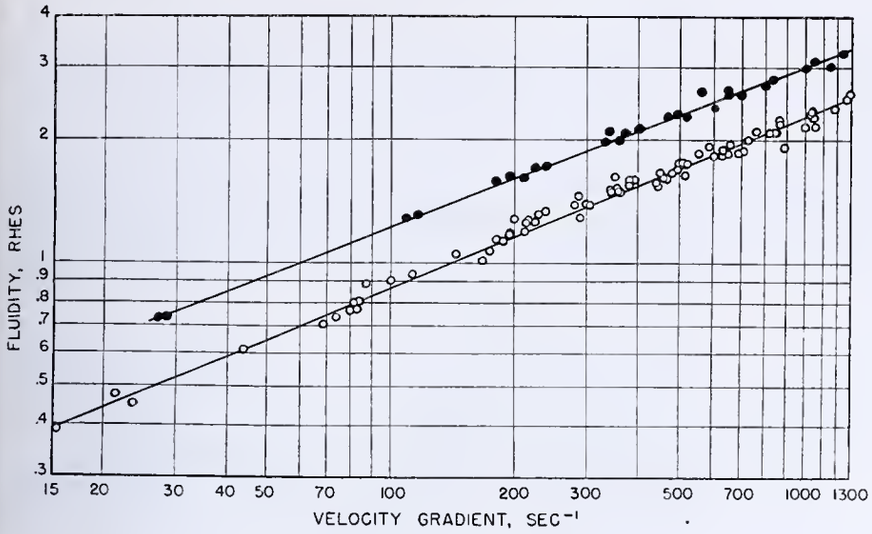

FIGURE 5. RELATION OF FLUIDITY OF 0.5 PER CENT CUPRAMMONIUM SOLUTIONS OF COTTON CELLULOSE TO VELOCITY GRADIENT

●. Cotton cellulose 1112
○. Cotton cellulose 300

solution, when measured in different types of instruments, or in the case of capillary viscometers, with capillaries of different radii. The other has to do with differences in results obtained in the identical instrument, but depending on the particular conditions of measurement—i. e., concentration of solution, total pressure, etc. Both influences trace their origin to a common source, the velocity gradient in the capillary during flow. In the case of Newtonian solutions the measured fluidity is independent of the velocity gradient. However, this is not the case with anomalous solutions.

In capillary viscometers the solution flows through a given capillary at different velocities, most rapidly at the center and slowest near the capillary wall. The solution in contact with the wall is essentially at rest. Therefore, there is always a gradient of velocity—i. e., a change in velocity per unit of distance, taken radially in the capillary. In 1929 Kroepelin (*14*) derived the following equation for mean velocity gradient $\bar{G}$, in capillaries:

$$\bar{G} = \frac{8V}{3\pi r^3 t} \qquad (6)$$

where V is the volume of solution, flowing in time t through a capillary of radius r. It is readily seen that $\bar{G}t$ becomes a constant for a given instrument from which $\bar{G}$ may be easily computed from observed values of t. [As Philippoff (*18*) has shown, $\bar{G}$ is not strictly the true mean velocity gradient in anomalous solutions, but a close approximation.]

Kroepelin showed that when the relative viscosities of solutions of caoutchouc in benzene, measured in capillaries of widely different radii, were plotted in linear coordinates against the mean velocity gradient they all fell on a smooth curve which rose rapidly as the gradient approached zero. Before plotting in this way, widely different results were obtained, depending on the capillary radii. This method of plotting, therefore, eliminated the instrument effect by permitting the results to be read off at some common velocity gradient. He showed, further, that when the data were plotted on double logarithmic coordinates the points fell on an apparently straight line over a 100-fold range of velocity gradient.

In view of the difficulties being experienced with the anomalous behavior of solutions of cellulose from raw cotton, it seemed desirable to try the application of Kroepelin's technique. The results of such a test are shown in Figure 4, where the fluidity values from Table I are plotted on double logarithmic coordinates. The fluidities of the glycerol solution and mineral oil are seen to be independent of the velocity gradient, as would be expected for Newtonian liquids. On the other hand, the two cotton cellulose solutions show fluidities falling on a linear curve inclined upward with increasing velocity gradients. By interpolation of the individual fluidities for the different liquids or solutions in Figure 4 parallel to the line representing their logarithmic regression on velocity gradient, to a velocity gradient of 500 cm. per second per centimeter, or sec.$^{-1}$, the fluidities in the right-hand column of Table I were obtained.

In order to extend the results over a wider range of velocity gradients, portions of two different samples of cotton cellulose were dissolved to give approximately 0.5 per cent solutions. The hydrostatic head was then decreased and augmented externally, as previously described, to change the rates of flow and in turn the mean velocity gradients. The results, covering a range of mean velocity gradients in one case from 15.3 to 1295 and in the other from 27 to 1244 sec.$^{-1}$, are plotted in Figure 5.

The observations are scattered somewhat, which is believed to be due, principally, to slight inaccuracies in reading short time intervals with the stop watch, to slight variations in concentration due to the hygroscopic nature of cellulose, and to the relatively very large influence of very slight variations of concentration. However, the results seem to indicate rather unmistakably that the observations, when plotted in this way, are practically linear over the range of velocity gradients employed. [According to Philippoff (*17*, *18*) the curves will, at a sufficiently low velocity gradient, eventually become parallel with the velocity gradient axis.] This conclusion has been confirmed also on a large number of individual curves on many samples of cotton cellulose, measured in the same range of mean velocity gradients. These results indicate, therefore, that serious instrument errors may occur in anomalous solutions if results are obtained at different velocity gradients. However, they may be eliminated by expressing the apparent fluidities or viscosities in terms of some common mean velocity

TABLE II. FLUIDITIES AND MEAN DEVIATIONS OF REPLICATES

(Determined with and without adjustment to a common velocity gradient, on different samples with viscometers of different capillary radii)

Sample No.	Viscometer No.	Capillary Radius	Mean Observed Velocity Gradient	Results Not Adjusted for Velocity Gradient		Results Adjusted to Velocity Gradient of 500 Sec.$^{-1}$	
				Fluidity	Mean deviation	Fluidity	Mean deviation
		Cm.	*Sec.*$^{-1}$	*Rhes*	*Rhe*	*Rhes*	*Rhe*
1270	67	0.0717	550	2.58	0.055	2.53	0.000
	68	0.0713	502	2.47		2.53	
1282	51	0.0719	460	2.12	0.065	2.25	0.015
	52	0.0702	410	1.99		2.22	
1300	51	0.0719	462	2.12	0.120	2.25	0.070
	52	0.0702	392	1.88		2.11	
1307	69	0.0722	535	2.84	0.100	2.83	0.055
	70	0.0710	490	2.64		2.72	
1321	64	0.0713	447	2.15	0.025	2.33	0.010
	65	0.0722	484	2.20		2.31	
1325	51	0.0719	508	2.34	0.060	2.38	0.010
	52	0.0702	461	2.22		2.36	
1329	64	0.0713	385	1.85	0.160	2.17	0.055
	65	0.0722	474	2.17		2.28	
1350	52	0.0702	518	2.54	0.055	2.55	0.030
	53	0.0710	547	2.65		2.61	
1362	70	0.0710	439	2.38	0.085	2.57	0.020
	71	0.0737	538	2.55		2.53	
1369	52	0.0702	402	1.82	0.130	2.03	0.050
	66	0.0715	491	2.08		2.13	
				Mean	0.086		0.032

gradient. The extent of such improvement, where capillaries of slight variations of radius are used, can be seen by reference to Table II, where replicate results are adjusted to a velocity gradient of 500 sec.$^{-1}$.

Relation of Anomalous Behavior to Molecular Size

However, the problem is more complicated than that involving merely instrument errors, especially if it is desired that the fluidity or viscosity values shall be indicative of the molecular magnitude of the solute. In order to clarify this point, it will be necessary to consider briefly the explanation of anomalous behavior as given by several investigators.

Signer (*23*) was the first to throw light on the nature of anomalous behavior, through studies of flowing anisotropy of solutions of various long-chain molecules. Signer's results indicated that the degree of optical anisotropy is a measure of the orientation of the extended filamentous molecules in the solution and that the failure of such solutions to obey the Poiseuille law is due to the energy required to orient these molecules.

Staudinger (*24*) accepts Signer's explanation and claims furthermore that anomalous flow is shown only by linear, but not spherical, colloids and in linear colloids only when the length of the molecule exceeds about 3000 Å. In the case of cellulose, this corresponds to a degree of polymerization of about 585 or a molecular weight of, roughly, 100,000. In solutions of linear molecules of shorter length the influence of Brownian motion is sufficient to counteract and prevent appreciable orientation.

Robinson (*21*) presents a dynamic picture of the extended molecules flowing through a capillary tube as being turned end-over-end by the gradient, rotating relatively rapidly when their long axes are in positions across the line of flow and relatively very slowly when in positions more nearly parallel to the line of flow. The result at any given moment is a statistical distribution of the axes in which the proportion of molecules aligned with or near the line of flow is larger the greater the gradient.

According to this picture, then, the apparent fluidity or viscosity depends on the degree of orientation of the extended molecules. Beginning with zero gradient and advancing to higher gradients, the apparent fluidity is almost constant for slight changes, because of the predominance of the Brownian influence, after which it increases rapidly because of the overtaking and predominance of the orienting influence. Finally, at very high gradients in which the long molecules are statistically essentially aligned in the direction of flow, the fluidity levels off again and remains practically constant with further increase in velocity gradient. The S-shaped curve of viscosity thus traced has been formulated by Philippoff (*17*) according to the equation:

$$\eta' = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + P^2/\gamma^2} \qquad (7)$$

in which η' is the apparent viscosity at any intermediate existing velocity gradient, η_∞ is a minimum limiting viscosity at high velocity gradients, η_0 is a maximum limiting viscosity at small gradients, approaching zero, P is the pressure, and γ is a material constant comparable to a modulus of elasticity.

It is obvious from the above discussion that in anomalous solutions η_0 is the viscosity value comparable to the viscosity of Newtonian solutions—i. e., the condition of random distribution of molecular axes.

Methods for Approximating η_0

Although a number of investigators have given special attention to the viscosity of long-chain polymers of varying axial ratios at minimum as well as at maximum orientation (*15*, p. 271 *et seq.*), the theory is still in the process of development. Thus far it has not yielded an adequate expression which will permit extrapolation to zero velocity gradient from a region of partial orientation.

Several empirical procedures have been suggested, with the aid of which the authors claim to be able to extrapolate viscosity to or near zero velocity gradient.

One of the most ingenious and interesting of these is that suggested by Williamson (*29*) in connection with the characteristics of paints. Williamson derives the equation:

$$\eta_0 = \frac{f}{s} + \eta_\infty \qquad (8)$$

in which f is the yield value corresponding to infinite rate of shear, s is the curvature of the rate of shear-stress curve at f, and η_∞ is the viscosity at infinite rate of shear. Williamson concluded that the values of f and η_∞ could be determined directly from a rate of shear-stress curve in which f is the intercept on the stress axis of the asymptote of the curve and η_∞ is the reciprocal of the slope of the asymptote. The value of s must be determined by a number of observations at different points below the critical value of stress where the curve turns toward zero rate of shear.

In the results shown in Figure 5, there is no evidence that the fluidity is approaching a constant maximum value at the highest velocity gradient used, even though the stress required to give this velocity gradient was far above that at which the rate of shear-stress curve is noticeably curvalinear. This means that even though the asymptote appears to be derivable from actual observations, actually in the case of the cellulose solutions it is not.

Staudinger (*25*) considers that if dilute enough solutions are employed the anomalous effect can be greatly reduced. Thus, in eucolloids—e. g., cellulose having a degree of polymerization above 500—the anomalous behavior is much greater in gel solutions than in sol solutions. He states further that not only must low concentrations be employed but low velocity gradients (near 100 sec.$^{-1}$) must be used in order that no, or only slight, orientation of the molecules can result.

However, a critical examination of data furnished by Staudinger (*24*, p. 508) and Staudinger and Sorkin (*27*) in comparison with data obtained in the present investigation and some published by Philippoff (*19*) does not seem to indicate that in the case of very highly polymerized substances very dilute solutions behave very differently from the more concentrated solutions with respect to velocity gradient. This can be seen by reference to Figure 6 in which the data, recomputed to intrinsic viscosities

$$[\eta] = \left(\frac{\eta_{sp}}{c}\right)_{c \to 0} \qquad (9)$$

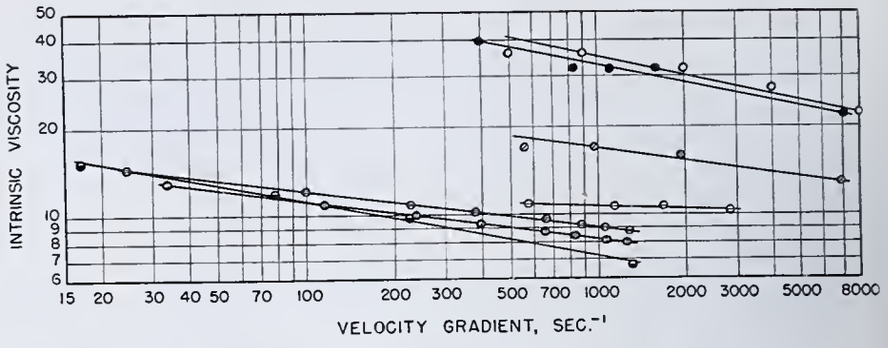

FIGURE 6. RELATION OF INTRINSIC VISCOSITY TO MEAN VELOCITY GRADIENT

○. 0.003 per cent nitrocellulose in butyl acetate, average d. p. 2650 (Staudinger and Sorkin)
●. 0.006 per cent nitrocellulose in butyl acetate, average d. p. 2680 (Staudinger)
⊗. 0.009 per cent nitrocellulose in butyl acetate, average d. p. 1285 (Staudinger)
◐. 0.03 per cent nitrocellulose in butyl acetate, average d. p. 955 (Staudinger)
◐. 0.5 per cent cotton cellulose 300 in cuprammonium solution, d. p. average about 2730
◐. 0.5 per cent cotton cellulose 1112 in cuprammonium solution, d. p. average about 2470
◒. 1 per cent nitrocellulose in butyl acetate (Philippoff)

where η_{sp} is the specific viscosity and c is the concentration in grams per 100 ml., are plotted in logarithmic coordinates against velocity gradient. The intrinsic viscosities for the data of Staudinger and of Staudinger and Sorkin were computed directly as the quotient of the natural logarithm divided by the concentration, the remainder with the aid of Philippoff's (*16*) eighth power modification of Baker's (*3*) equation described below. As can be seen, the slope of the 0.003 and 0.006 per cent solutions is almost identical with that for the 1 per cent solution computed from Philippoff's data and even greater than that for the 0.5 per cent cuprammonium solutions of cotton cellulose. This conclusion is in line with that of Fikentscher (*9*) who states, "Da er (pressure dependence) durch keine Aenderung des Lösungmittels oder der Messtemperatur oder der Messapparatur auch nicht bei niedrigen Konzentrationen vermeiden werden kann, ist es auch hier nötig, fur die Bestimmung der Eigenviskosität dieser Produkte eine bestimmte Schergeschwindigkeit bzw. einen bestimmten Ueberdruck festzulegen." If the theory given above of the influence of velocity gradient on the viscosity of anomalous solutions is correct there would seem to be no a priori reason why dilution should overcome it.

A second method suggested for approximating η_0 is based on Philippoff's (*16*) eighth power formula. Here again η_0 is expressed in the form of the quotient with the concentration, as the intrinsic viscosity,

$$[\eta] = \frac{8}{c}\left(\sqrt[8]{\eta_r} - 1\right) \qquad (10)$$

in which c is expressed preferably in weight per cent—i. e., grams of solute per 100 grams of solvent—and η_r is the relative viscosity. The intrinsic viscosity, $[\eta]$, is, except for the unit in which concentration is expressed, equivalent to Staudinger's expression:

$$\lim. \ (\eta_{sp}/c)_{c \to 0}$$

and since this expression is used by Staudinger for determination of molecular weight there is real advantage in obtaining the result in this form.

Hess and Philippoff (*12*) obtained very good agreements of the value of $[\eta]$ of technical collodion in butyl acetate solutions, in the wide range of concentrations from 0.05 to 15.00 weight per cent, except that the values for concentrations of 1 per cent and less were about 4 per cent higher than those for higher concentrations. More recently Philippoff and Krüger (*20*) found this equation to give good reproducibility of $[\eta]$ in viscose solutions, varying in concentration from 0.02 to 11.99 per cent, except that here again the values of $[\eta]$ for concentrations of 1 per cent or less averaged higher, and in this case about 17 per cent. Both sets of solutions were highly anomalous, so that the success of this procedure is remarkable.

Application of Philippoff's equation to the results from undeteriorated cellulose shows a different result; however, it does not by any means overcome the anomalous effect. Table III gives data for 0.5 per cent cuprammonium solutions of two cottons, and for comparison, because of the much lower velocity gradients included, data by Philippoff (*19*) on 1 per cent nitrocellulose in butyl acetate. The latter have been recomputed to the same units, the absolute viscosity of butyl acetate in c. g. s. units at 20° C. being taken as 0.00727 poise. The very pronounced influence of velocity gradient is clearly shown. Even at the lowest velocity gradient attained by Philippoff, 0.0238 sec.$^{-1}$, the intrinsic viscosity has not yet become constant. It is scarcely possible to exceed this lower limit of velocity gradient with even the most specialized capillary viscometers. It is therefore evident that η_0 cannot be evaluated by this technique.

Still a third method for the attainment of η_0 has been suggested by Staudinger and Sorkin (*27*), based on the extrapolation of the reciprocal of Staudinger's value

$$\lim. \ (c/\eta_{sp})_{c \to 0}$$

against velocity gradient, using linear coordinates. In dealing with a series of four nitrocelluloses, varying in degree of polymerization from 310 to 2650, they obtained, between the velocity gradients 500 and 16,000 sec.$^{-1}$ almost linear, slightly inclined curves, which were practically parallel and displaced from one another in the order of the degree of polymerization.

In view of the relationship indicated in Figure 6 it might be anticipated that this device could not be used for extrapolation to zero velocity gradient. Figure 7 shows that this is indeed the case. The reciprocals of the intrinsic viscosity are nearly linear with decreasing velocity gradient to approximately 500 sec.$^{-1}$, whereupon they turn down sharply and extrapolation by this procedure to zero velocity gradient becomes impossible.

Thus it is evident that none of the methods discussed above is adequate for evaluating η_0. A means for interpolating viscosity from intermediate to zero velocity gradients will no doubt be worked out eventually. Meanwhile the best procedure for treating fluidity or viscosity data, measured at various velocity gradients, seems to be to interpolate or extrapolate to some convenient velocity gradient. Since the logarithmic curve relating fluidity and viscosity, as well as intrinsic viscosity and Staudinger's value $(\eta_{sp}/c)_{c \to 0}$, to velocity gradient seems to be practically if not actually linear (Figure 6) well over the range for any ordinary measurement, it does not seem urgent that the viscosity measurements be made at the lowest possible velocity gradients—i. e., at 100 sec.$^{-1}$—as suggested by Staudinger and Sorkin (*27*). It is desirable, however, that the results of measurements upon anomalous solutions be reported in terms of some convenient standard gradient. Since a large part of the data recorded in the literature has

Table III. Relation of Intrinsic Viscosity, Computed by Philippoff's Eighth Power Formula, to Velocity Gradient

Product	Solvent	Concentration of Solution	Diameter of Capillary	Length of Capillary	Velocity Gradient	Absolute Viscosity	Relative Viscosity	Intrinsic Viscosity
		%	*Cm.*	*Cm.*	*Secs.*$^{-1}$	*Poises*		
Cotton 300	Cuprammonium	0.5	0.0717	2.5	1295	0.386	31.8	8.67
		0.5	0.0717	2.5	1048	0.427	35.1	8.96
		0.5	0.0717	2.5	873	0.449	36.9	9.14
		0.5	0.0717	2.5	637	0.530	43.6	9.65
		0.5	0.0722	2.5	353	0.660	54.2	10.37
		0.5	0.0722	2.5	212	0.795	65.4	10.98
		0.5	0.0722	2.5	82.2	1.300	107.0	12.69
		0.5	0.0722	2.5	10.7	3.677	302.3	16.67
Cotton 1112	Cuprammonium	0.5	0.0714	2.5	1156	0.321	27.2	8.19
		0.5	0.0714	2.5	1015	0.334	27.5	8.22
		0.5	0.0714	2.5	806	0.369	30.3	8.53
		0.5	0.0714	2.5	608	0.417	34.3	8.90
		0.5	0.0702	2.5	368	0.483	39.7	9.36
		0.5	0.0702	2.5	224	0.585	48.1	9.97
		0.5	0.0702	2.5	108	0.781	64.2	10.93
		0.5	0.0702	2.5	27	1.374	113.0	12.90
Nitrocellulose (Philippoff)	Butyl acetate[a]	1.0	0.030	12.0	1300	0.95	130.7	6.70
		1.0	0.030	12.0	224	4.23	582	9.74
		1.0	0.030	12.0	78	10.5	1,445	11.86
		1.0	0.300	12.0	17.06	37.9	5,215	15.38
		1.0	0.300	12.0	1.826	206	28,350	20.82
		1.0	0.300	12.0	0.181	755	103,850	25.92
		1.0	0.300	12.0	0.0238	1290	177,500	28.24

[a] Absolute viscosity of butyl acetate at 20° C. was taken as 0.00727 poise.

been obtained probably at velocity gradients between 100 and 1000 sec.$^{-1}$, a convenient value might be intermediate, say at 500 sec.$^{-1}$. This would come within the range recommended by Schulz (*22*).

Calculation of Molecular Weight of Cellulose

As is well known, Staudinger (*24*) computes molecular weights with the aid of the formula

$$M = \frac{\eta_{sp}/c}{K_m} \qquad (11)$$

[As has been beautifully shown by Fordyce and Hibbert (*10*) as well as by others, this equation requires modification, especially for comparatively short-chained molecules. However, in the range of chain length discussed in the present paper the modification has an insignificant effect.] The value η_{sp}/c is the limiting value as the concentration approaches zero and K_m is a constant which has been evaluated from substances of relatively low molecular weight. The value of K_m found with Newtonian or near-Newtonian solutions will not apply, of course, to highly anomalous solutions, as is readily evident from Figure 6. A requirement for the use of Staudinger's formula for highly anomalous solutions would seem to be the determination of the molecular weight by an independent method, such as the ultracentrifuge, as was done by Kraemer (*13*), or less preferably by osmotic measurements such as those carried out by Staudinger and Schulz (*26*). The intrinsic viscosity of the same product determined then interpolatively at a standardized velocity gradient would provide a value of K_m suitable for molecular weight determinations at this velocity gradient. Whether K_m would prove to be strictly constant for different members of a homologous series under these conditions is a matter requiring further study.

Assuming for the moment that the above relationships have been determined, that a velocity gradient of 500 sec.$^{-1}$ has been decided upon, and that at this velocity gradient Kraemer's (*13*) value of $DP/[\eta] = 260$ can be accepted for cellulose in cuprammonium solution, the molecular weight of a cotton cellulose can be computed from the fluidity of 0.5 per cent solutions in the following manner:

First, Philippoff's equation may be partially evaluated:

$$[\eta] = \frac{8}{c}\left(\sqrt[8]{\frac{1/F_s}{1/F_o}} - 1\right) = \frac{8}{0.5}\left(\sqrt[8]{\frac{82.25}{F_s}} - 1\right) = 16\,(1.735\,\sqrt[8]{1/F_s} - 1) \qquad (12)$$

where F_s is the fluidity of the solution and F_o that of the solvent (equals 82.25 rhes). The intrinsic viscosity having been computed the degree of polymerization, DP, and molecular weight, M, become, respectively

$$DP = 260\,[\eta] \qquad (13)$$

$$M = 162\,(260\,[\eta] = 42{,}120\,[\eta] \qquad (14)$$

Thus, a cellulose in 0.5 per cent cuprammonium solution giving a fluidity of 0.80 rhe at a velocity gradient of 500 sec.$^{-1}$ would have an intrinsic viscosity of 12.6, a degree of polymerization of 3280, and a molecular weight of 531,000.

Summary and Conclusions

In a study of methods of expressing the results of moderately to highly anomalous solutions, such as 0.5 per cent cuprammonium solutions of undeteriorated cotton cellulose, the velocity gradient at which the measurement is made was found to be an important factor.

The results of the present study as well as a critical examination of the literature do not suggest any practical means for overcoming or avoiding the anomalous effect, or of obtaining results equivalent to those for Newtonian liquids.

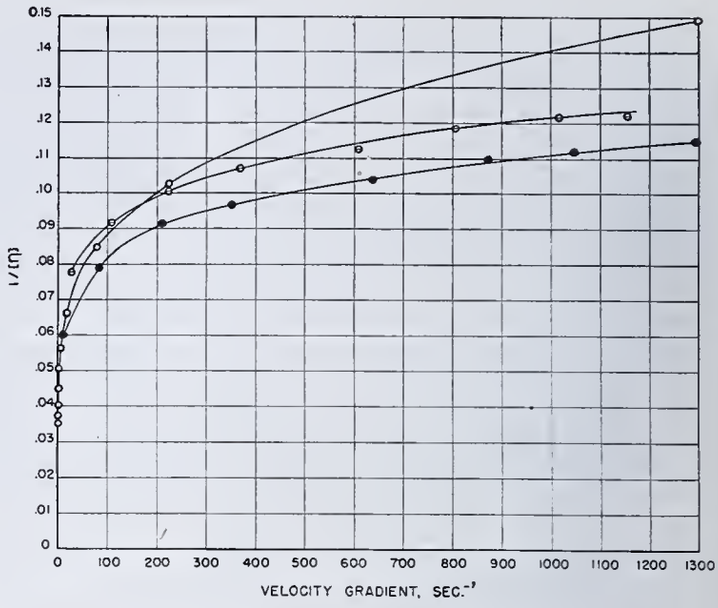

FIGURE 7. RELATION OF RECIPROCAL OF INTRINSIC VISCOSITY TO VELOCITY GRADIENT

○. 1 per cent nitrocellulose in butyl acetate (Philippoff, *19*)
⊖. 0.5 per cent cotton cellulose 1112 in cuprammonium hydroxide
●. 0.5 per cent cotton cellulose 300 in cuprammonium hydroxide

The most promising procedure for anomalous solutions consists in obtaining fluidity or viscosity readings at several velocity gradients and then interpolating the results logarithmically to some common mean velocity gradient. Methods for varying the mean velocity gradient are suggested.

A mean velocity gradient of 500 sec.$^{-1}$ is recommended as convenient of attainment in ordinary capillary viscometers and probably representative of gradients for which results of anomalous solutions are recorded in the literature.

The adoption of a common mean velocity gradient for expression of the results of anomalous solutions will not only eliminate instrument errors but will provide a unique value for any given substance. Methods are suggested for relating this to the molecular weight.

Acknowledgment

The writer is indebted to James H. Kettering for much assistance with the fluidity measurements used in this paper.

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Colorimetric Determination of Copper with Ammonia

A Spectrophotometric Study

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THE determination of copper, which depends upon the production of the intense blue color of the cupric ammonia complex when ammonia is added to a solution of a cupric salt, is one of the oldest colorimetric methods.

Among the first workers to use it was Heine (*11*) in 1830, followed by Dehms (*6*) and Bischof (*3*). More recently it has been applied to the determination of copper in peas (*4*); in blast slags and tailings (*1*); in preserves (*19*); in marine organisms (*17*); in the human body (*2*); in iron, steel, and slag (*20*); in vegetables (*9*); and in rubberized fabrics (*18*). Snell (*21*) has claimed that the method is most sensitive when 0.01274 mg. of copper per milliliter is present and that at this concentration the addition of 0.000016 mg. of copper can be detected. It is doubtful, however, if the method is as sensitive as these figures would imply, for Yoe and Crumpler (*27*), using a roulette comparator, found that a solution containing 8 p. p. m. of copper could be distinguished only with difficulty from one containing 7 or 9 p. p. m. and with certainty from one containing 6 or 10 p. p. m.

The purpose of the work described in this paper was to make a critical study of this method by means of the photoelectric recording spectrophotometer (*14*), with particular attention to the effect of diverse ions upon the color system. Similar studies of other colorimetric methods have recently been made (*5, 7, 8, 12, 13, 22, 24*).

Apparatus and Solutions

All spectrophotometric measurements in the present work were made at Purdue University with the instrument used by the writer in two previous investigations (*13*).

A standard stock solution of cupric sulfate pentahydrate, each milliliter of which contained 4 mg. of copper, was made by dissolving 15.7160 grams of the thrice recrystallized salt in redistilled water, adding 1 ml. of concentrated sulfuric acid, and accurately diluting to 1 liter.

Ammonium hydroxide solutions of various concentrations, such as 1, 2, 3, 4, and 6 *M*, were made by suitable dilution of the concentrated solution of specific gravity 0.90.

Standard solutions of the diverse ions were prepared from the chloride, nitrate, or sulfate salts of the cations and from the sodium, potassium, or ammonium salts of the anions. Redistilled water was used in all cases. Each milliliter contained 10 mg. of the ion in question.

To produce the color system 5 ml. of the standard copper solution in a 100-ml. volumetric flask were just neutralized with 15 *M* ammonium hydroxide, diluted to the mark with 3 *M* ammonium hydroxide (*25*), and thoroughly shaken.

The spectral transmission curves were determined for a solution thickness of 1.961 cm. and a spectral band width of 10 mμ. Compensation for the absorption of the glass cell and solvent was obtained by placing in the rear beam of light a similar cell filled with 3 *M* ammonium hydroxide.

That the color reaction may be reproduced to a high degree of precision is shown by the fact that eleven solutions of the cupric ammonia complex, each containing 200 p. p. m. of copper and prepared by the above procedure, gave transmittancies at 620μ (the peak of the absorption band), the average deviation of which from the mean was only 0.1 per cent.

Conformity to Beer's Law

That Beer's law is followed by the color system is shown by the fact that a straight line resulted when the logarithms of the observed transmittancies at 620 mμ for six solutions, containing from 40 to 600 p. p. m. of copper, were plotted against the respective concentrations. This is in agreement with the work of other investigators (*16, 26, 27*).

Effect of Ammonium Hydroxide

Different workers have used various concentrations of ammonium hydroxide to produce the cupric ammonia complex. Yoe (*25*) used 3 *M*, Heath (*10*) 2.1 *M*, and Snell (*21*) 1.5 *M*. Yoe and Crumpler (*27*) have pointed out that ammonia solutions show an appreciable absorption of light in the visible region.

In a study of the effect upon the color of the concentration of ammonium hydroxide, the transmission curves produced by a series of solutions, each containing 200 p. p. m. of copper, but made by use of 1 *M*, 2 *M*, 3 *M*, 4 *M*, 6 *M*, and Snell's approximately 1.5 *M* ammonium hydroxide, were compared. In each case the corresponding concentration of ammonium hydroxide was used in the rear cell. The curves show that the hue gradually changes as the concentration of ammonium hydroxide varies. Both the maximum absorption and the wave length of maximum absorption increase as the concentration increases. This is in accordance with the findings of Weigert (*23*) and of Yoe and Barton (*26*) who used only two concentrations of ammonium hydroxide. Therefore, it is necessary that the concentration chosen for the determination be used throughout in making all the unknown as well as standard solutions which are to be used for comparison. In the present work the 3 *M* solution (*25*) was selected. Because of the high concentration of ammonia, no attempt was made to determine pH values.

TABLE I. EFFECT OF DIVERSE ANIONS

(20 mg. of copper in 100 ml. of solution)

Ion	Concentration	Apparent Change in Copper Concentration	Approximate Limiting Concentration
	P. p. m.	%	*P. p. m.*
Acetate	500	Negligible	...
Arsenate	500 (As)	Negligible	...
Arsenite	500 (As)	Negligible	...
Borate	250 (B_2O_3)	Negligible	...
Benzoate	500	Negligible	...
Bromide	500	Negligible	...
Carbonate	600	Negligible	...
Chlorate	500	Negligible	...
Chloride	500	Negligible	...
Chlorostannate	500 (Sn)	Precipitates	
	100 (Sn)	+6.0	0
Chlorostannite	100 (Sn)	Precipitates	0
Citrate	500	−6.5	
	100	−2.0	100
Cyanide	350	Decolorized	
	100	−27.0	0
Dichromate	100 (Cr)	Change in hue	0
Fluoride	500	Negligible	...
Formate	500	Negligible	...
Iodide	500	Negligible	...
Molybdate	500	+2.5	
	200	+2.0	200
Nitrate	500	Negligible	...
Nitrite	500	Negligible	...
Orthophosphate	500 (P_2O_5)	Negligible	...
Oxalate	500	Negligible	...
Perchlorate	500	Negligible	...
Pyrophosphate	100	Change in hue	0
Salicylate	100	Change in hue	0
Silicate	500 (SiO_2)	−2.5	
	100 (SiO_2)	Negligible	250
Sulfate	500	Negligible	...
Sulfite	500	Negligible	...
Tartrate	500	−5.4	
	200	−2.3	100
Thiocyanate	500	Negligible	...
Thiosulfate	500	−8.7	
	400	−2.3	
	300	Negligible	350
Tungstate	300	Turbidity	
	200	+6.0	
	100	Negligible	100
Vanadate	50 (V)	Turbidity	0

Stability of the Color

Six solutions, containing 40, 80, 120, 400, and 600 p. p. m. of copper, respectively, in 3 *M* ammonium hydroxide, were stored in glass-stoppered Pyrex bottles and allowed to stand in diffuse light. Curves made at intervals for these solutions gave no evidence whatever of fading or of other change in color over a period of six weeks. Lack of time prevented tests over a longer period. Apparently the color is stable indefinitely. Such marked stability makes possible the use of a series of permanent standards, which must, however, be kept tightly stoppered to prevent loss of ammonia. Any action of the ammonia on the glass to produce turbidity is reduced to a minimum by using Pyrex containers.

Effect of Anions

In the ion-interference studies the curve produced by the standard solution containing 200 p. p. m. of copper in 3 *M* ammonium hydroxide was compared with the curve produced by a similar copper solution containing in addition a known weight of the diverse ion. From the transmittancies at 620 mμ of the standard solution and of each of the other solutions and from the known copper concentration of the standard solution, the apparent concentration of copper in each of the solutions containing diverse ions was calculated by the aid of a special color slide rule. The difference between this value and the actual concentration multiplied by 100 and divided by the actual concentration gave the percentage error. The calculation is based upon the formula which expresses Beer's law

$$T_2 = T_1^{\frac{c_2}{c_1}}$$

where T_1 represents the transmittancy, expressed as a decimal, for the solution of concentration c_1, and T_2 the transmittancy for the solution of concentration c_2. In accordance with the procedure followed in two former studies by the writer (*13*) and by other workers at Purdue University (*5, 7, 8, 12, 22, 24*), a 2 per cent error was set as the maximum allowable for negligible interference. Although visual methods of color comparison often have a precision of not less than 5 per cent, it was thought advisable to set a lower figure to provide for other possible factors including the effect of the extinction coefficient.

The interfering anions may be divided into four general classes: those, such as citrate, cyanide, tartrate, and thiosulfate, which form complexes with copper without a change in hue; those, such as pyrophosphate and salicylate, which form complexes with a change in hue; those, such as dichromate, which cause a change in hue because of their own color; and those, such as chlorostannate, chlorostannite, tungstate, and vanadate, which cause turbidity because of their own hydrolysis or the precipitation of copper salts.

In Figure 1, curve 2 shows the effect of 100 p. p. m. of cyanide ion, curve 3 the effect of 500 p. p. m. of thiosulfate ion, curve 4 the effect of 200 p. p. m. of tungstate ion, and curves 5 and 6 the effects of 100 and 500 p. p. m. of chromium as dichromate ion.

The effects of the common anions and their approximate limiting concentrations are listed in Table I.

Effect of Cations

Cobaltous and nickelous ions are the only common cations which really interfere. They form soluble, colored ammonia complexes, causing a change in hue. In Figure 2, curves 2 and 4 show the effects of 100 and 500 p. p. m. of nickelous ion and curves 3 and 5 the effects of 25 and 100 p. p. m. of cobaltous ion. Although silver, cadmium, and zinc ions form am-

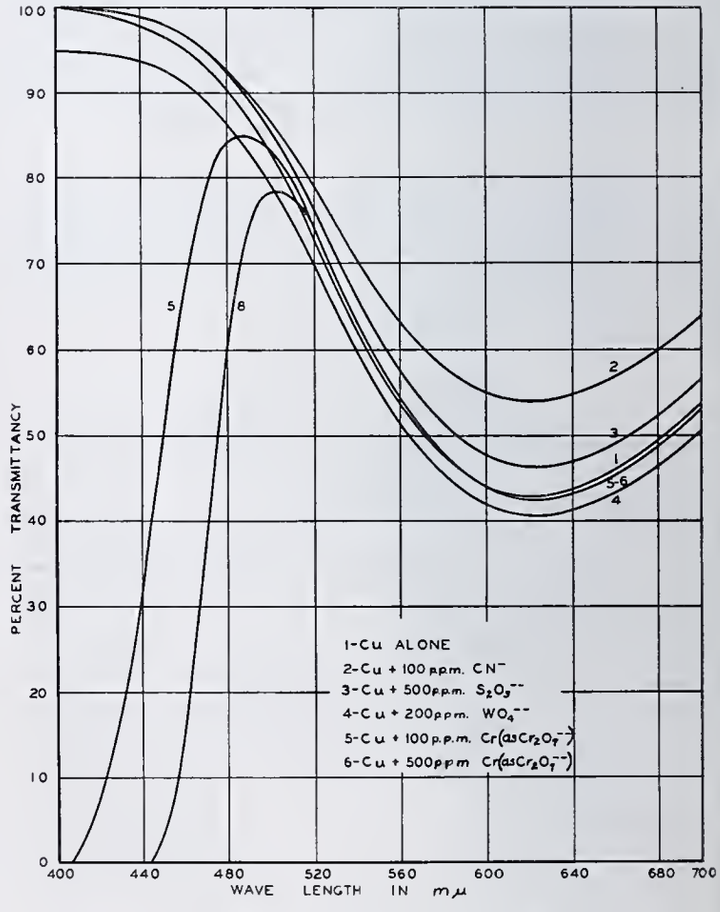
PERCENT TRANSMITTANCY
WAVE LENGTH IN mμ
1-Cu ALONE
2-Cu + 100 p.p.m. CN⁻
3-Cu + 500 p.p.m. S₂O₃⁻⁻
4-Cu + 200 p.p.m. WO₄⁻⁻
5-Cu + 100 p.p.m. Cr(as Cr₂O₇⁻⁻)
6-Cu + 500 p.p.m. Cr(as Cr₂O₇⁻⁻)

FIGURE 1. EFFECT OF ANIONS

20 mg. of copper with diverse ion diluted to 100 ml. with 3 *M* ammonium hydroxide. 1.961-cm. cell.

monia complexes, the latter are colorless and do not interfere. Barium, lead, and strontium ions precipitate as sulfates, and aluminum, antimonous, beryllium, bismuth, chromic, ferric, ferrous, magnesium, manganous, mercuric, mercurous, thorium, uranyl, and zirconium ions precipitate as hydroxides or basic salts in the alkaline solution. In the course of the determination (*10*, *21*, *25*), however, fuming down with sulfuric acid, followed by addition of ammonium hydroxide and filtration, would remove all these ions and so prevent interference.

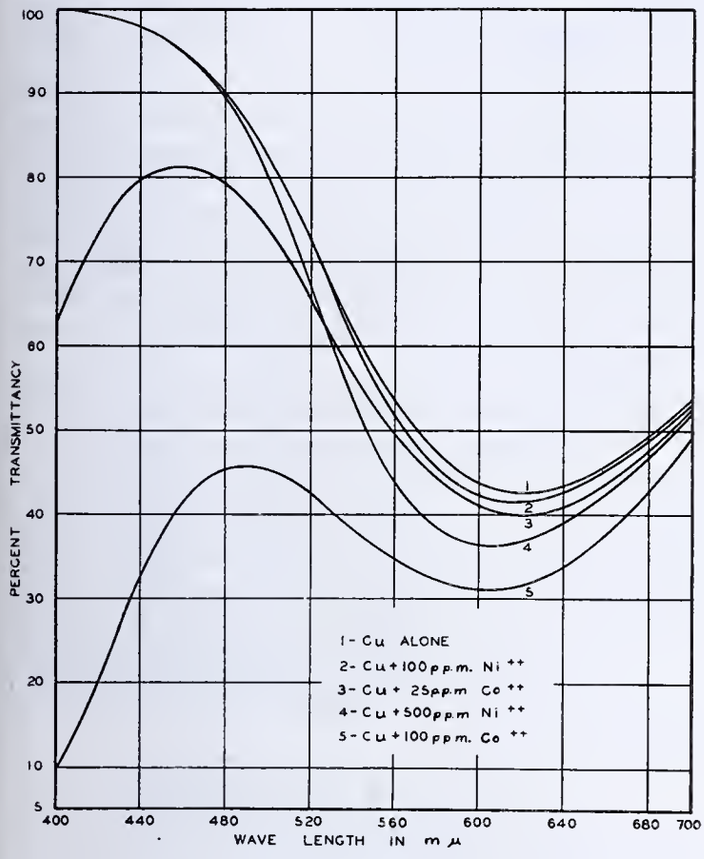

FIGURE 2. EFFECT OF CATIONS
20 mg. of copper with diverse ion diluted to 100 ml. with 3 *M* ammonium hydroxide. 1.961-cm. cell.

The ammonium ion when introduced as the sulfate has a negligible effect upon the color, but 200 p. p. m. of ammonium ion as chloride cause an error of +2 per cent in the concentration of copper, while 500 p. p. m. of ammonium ion as chloride cause an error of +3.3 per cent. For larger amounts, such as 1000 p. p. m. and 1500 p. p. m., this error remains constant at +3.3 per cent. Therefore, not more than 200 p. p. m. of ammonium ion as ammonium chloride should be present. Milbauer and Stanek (*15*) have apparently erroneously stated that the intensity of the color decreases with increasing amounts of ammonium chloride. The transmission curves prove that there is an increase in intensity. Yoe and Barton (*26*) have obtained a similar result.

In Table II are listed the effects and approximate limiting concentrations of the common cations which do not cause precipitation.

Summary

A spectrophotometric study shows that the ammonia method for the colorimetric determination of copper is very satisfactory with few limitations. The procedure of Yoe (*25*) is recommended.

The concentration of the ammonium hydroxide used for dilution must be carefully controlled because of the ability of the ammonia solution to absorb light. The same concentration must be used for the standards as for the unknown solutions. A 3 *M* solution is recommended.

The color system follows Beer's law.

The color is stable in diffuse light for at least six weeks and undoubtedly for a much longer time. The use of a series of permanent standards is therefore possible.

The color reaction may be reproduced to a high degree of precision.

A study of the effect of sixty common ions shows that only a few, especially cobaltous, nickelous, and dichromate, seriously interfere with the color and that a considerable number of others cause precipitation or turbidity, but in the course of the determination this latter group would be removed.

TABLE II. EFFECT OF DIVERSE CATIONS
(20 mg. of copper in 100 ml. of solution)

Ion	Concentration	Apparent Change in Copper Concentration	Approximate Limiting Concentration
	P. p. m.	%	*P. p. m.*
Ammonium	400	Negligible[a]	..
Cadmium	500	Negligible	..
Calcium	500	Negligible	..
Cobaltous	25	Change in hue	0
Lithium	250	Negligible	..
Nickelous	50	Change in hue	0
Potassium	500	Negligible	..
Silver	500	Negligible	..
Sodium	600	Negligible	..
Zinc	500	Negligible	..

[a] Provided chlorides do not exceed 400 p. p. m.

Acknowledgments

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Determination of Soluble Silica in Water

A Photometric Method

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This method measures the molybdenum blue color developed by reducing the yellow silicomolybdate complex with sodium sulfite at suitable pH. It requires only 4 minutes for a test, a 10-ml. sample, and gives an accuracy on par with gravimetric analysis. Phosphate, alkalinity, tannins, iron, and other ions present in natural and boiler waters in addition to color, temperature, and color progression, offer no practical interference. The work was completed with a Klett-Summerson photoelectric photometer, but the method can be employed with any similar instrument.

DIENERT and Wandenbulke (*2*) have shown that small concentrations of soluble silica can be determined colorimetrically by measurement of the yellow color that develops on the interaction of soluble silica and ammonium molybdate in acidic solution. The yellow color is stated to be a complex of composition $H_8Si(MoO_7)_6H_2O$ (*2*, *5*). Since the appearance of this method, Thayer (*12*) has shown that it is subject to interference from phosphate and iron, due to formation of phosphomolybdate and iron silicomolybdate. Numerous other investigators have used essentially this same method for the determination of silica, most of them using Nessler or Hehner tubes for comparison or different types of colorimeters (*6*, *7*, *9*) such as Duboscq (*10*). An exhaustive survey of the literature has failed to reveal any mention that tannins in natural and boiler waters interfere with this method. This interference was noted in this laboratory while attempting to apply the Dienert-Wandenbulke method using the Klett-Summerson photoelectric photometer. Natural and boiler waters gave higher results than gravimetric analysis.

In order to investigate this further, tannins of two different classes, pyrocatechol and pyrogallol, were investigated for their reaction with molybdate by the Dienert-Wandenbulke procedure applied to the Klett-Summerson photometer and the Taylor visual comparator. The results recorded in Table I show that both types of tannins interfere with this method, giving high silica equivalence. The pyrogallol type per unit weight forms a more intense yellow complex with molybdate than the pyrocatechol type and therefore gives higher silica equivalence. This work showed conclusively that the Dienert-Wandenbulke test could not be used for accurate work in waters having tannins present.

Isaacs (*4*) reported that reduction of the silicomolybdate complex by sodium sulfite to molybdenum blue of composition $Mo_3O_8.H_2O$ (*8*) in low acidity is not accompanied by color from phosphomolybdates. Bertrand (*1*) criticized the method, but Foulger (*3*) showed that phosphate, free from silica, gives no color with the reagent at the acidity recommended by Isaacs. The latter procedure was not found suitable for rapid photometric work, as it employs acetic acid at relatively high pH, which gives color progression too great for accurate work.

In the present study, the use of hydrochloric acid for development of the silicomolybdate complex at pH 2.4 to 2.7, followed by reduction by sodium sulfite, was found to be very satisfactory, giving adequate silica sensitivity and no practical interference from phosphate and tannins. The effect of pH on the formation of phosphomolybdate before reduction and the subsequent silica equivalence after reduction is given in Figure 1. As the acidity decreases above pH 2.7, the effect of phosphate becomes negligible, but the color development when silica is present requires more time and is accompanied by considerable color progression. The pH range of 2.4 to 2.7 before reduction is considered optimum and is the basis for this present method.

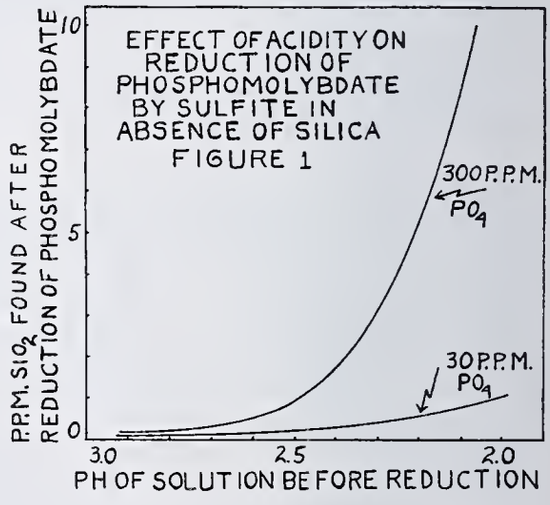

Table I. Interference of Tannins with the Dienert-Wandenbulke Method in the Absence of Silica

Pyrocatechol Class Tannin[a]				Pyrogallol Class Tannin[a]			
Tannin	SiO_2 equivalence, photometer	Tannin	SiO_2 equivalence, Taylor comparator	Tannin	SiO_2 equivalence, photometer	Tannin	SiO_2 equivalence Taylor comparator
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
2	0.4	2	1	2	0.7	2	1
10	1.2	5	1	10	3.0	4	2
50	6.4	10	2	50	16.8	10	4
100	14.8	25	6	100	36.6	25	15

[a] Commercial grades of silica-free tannins.

Reagents

Hydrochloric acid reagent, 0.248 *N*, 20 ml. of 38 per cent grade (1.19 specific gravity) acid per liter. Ammonium molybdate reagent, 102 grams of ammonium molybdate (grade 81 per cent MoO_3) per liter. Sodium sulfite reagent, 170 grams of sodium sulfite (grade 97 per cent, anhydrous) per liter. Sodium silicate solution, 102.0 mg. as silica per liter.

Procedure and Conditions of Test

The procedure uses 10 ml. of sample for analysis, 5 ml. of hydrochloric acid reagent, 5 ml. of ammonium molybdate reagent, and 10 ml. of sodium sulfite reagent. The sample is treated with the

Table II. Effect of Phosphate

Disodium Phosphate Present	Silica Found by Photometer		
	Present, 9.2 p. p. m.	Present, 24.7 p. p. m.	Present, 42.0 p. p. m.
P. p. m. PO_4	*P. p. m.*	*P. p. m.*	*P. p. m.*
20	9.3	24.6	42.3
40	9.4	24.8	42.2
100	9.4	24.6	42.2
200	9.5	24.6	41.5
500	9.0	23.0	40.8

Table III. Effect of Tannins[a]

SiO_2 Present	SiO_2 Found	SiO_2 Difference	Tannin Present		
			Pyrocatechol type	Pyrogallol type	Pure tannic acid
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
0	0.3	+0.3	200	..	..
	0.7	+0.7	400	..	..
	1.1	+1.1	600	..	..
	1.5	+1.5	1000	..	..
18.5	18.6	+0.1	..	..	..
	19.7	+1.2	200	..	..
	21.2	+2.7	400	..	..
	21.6	+3.1	600	..	..
	22.5	+4.0	1000	..	..
0	0.7	+0.7	..	200	..
	1.3	+1.3	..	400	..
	2.0	+2.0	..	600	..
	2.8	+2.8	..	800	..
	3.2	+3.2	..	1000	..
18.5	18.7	+0.2	..	..	..
	19.3	+0.8	..	200	..
	21.4	+2.9	..	400	..
	22.1	+3.6	..	600	..
	23.0	+4.5	..	800	..
	23.7	+5.2	..	1000	..
0	0.9	+0.9	..	..	200
	2.1	+2.1	..	..	600
	3.3	+3.3	..	..	1000
18.5	20.5	+2.0	..	..	200
	23.0	+4.5	..	..	600
	25.0	+6.5	..	..	1000

[a] All products were SiO_2-free. Pyrocatechol and pyrogallol types are 70% tannins; pure tannic acid is 100%.

acid and molybdate reagents and allowed to stand for approximately 1 minute. This gives the silicomolybdate complex time to develop completely, after which it is stable for 5 minutes. During this time interval, another sample is treated with the acid and sulfite reagents in addition to distilled water equivalent to the molybdate reagent, and is used to adjust the photometer to zero reference. When this operation is complete, the test sample is reduced by the sulfite reagent and tested at the end of 1 minute. The Klett-Summerson photometer has two photocells of the blocking type, a standard source of illumination, a sensitive galvanometer, color filters of average bands, a 21-cm. (14-inch) logarithmic scale, and operates with 110 volts alternating or direct current. Summerson (*11*) gives a detailed discussion of the photometer. The work was completed with a 13-mm. test tube to facilitate rapid routine analysis. The instrument also accommodates sample cell depths of 2.5, 10, 20, and 40 mm. The blue reduction color of the final silica test was found to absorb best in the spectral region 600 to 700 mμ. In this work, a 620 mμ filter (590 to 680) was employed.

With this procedure a standard curve was established from a series of points of known silica content. The curve is linear throughout the concentration range of 0 to 50 p. p. m. of silica used in this study. The curve was checked by other standard solutions prepared from pure silica fused in sodium carbonate, and hydrolyzed silicon tetrachloride. Hard-rubber bottles were used throughout this study to prevent contamination from glass bottles.

Effects of Ions and Conditions on the Method

Orthophosphate, a common constituent in boiler waters, does not interfere with the method, as shown in Table II. A comparison of results given in Figure 1 and Table II shows that phosphate in the absence of silica gives some silica equivalence, while in the presence of silica this effect is observed only with low silica content. In Figure 1, 300 p. p. m. of disodium phosphate as PO_4 exhibit 0.8 to 2 p. p. m. of silica between pH 2.7 and 2.4. In Table II, 300 p. p. m. of PO_4 in the presence of silica give as high as 0.2 p. p. m. of silica in low silica content and give low silica results on the order of 1 p. p. m. in high silica content. The reason for this phenomenon is not clear. It cannot be explained on the basis that the phosphate buffers the solution away from the correct pH range, as the buffering effect of this concentration of phosphate was found to be only 0.1 pH. It must in some way interfere with either the complete formation of the silicomolybdate complex or the reduction of this complex to molybdenum blue.

Tannin of two commercial varieties and pure tannic acid offer no practical interference, as Table III reveals. The effect of the tannins is different in the presence and absence of silica. The yellow tannin molybdate complex is not reduced by sulfite to molybdenum blue, but its color gives an additive effect to the silica present. Even in the most highly colored boiler waters, the tannin concentration is rarely over 200 p. p. m., which offers no practical interference to the method.

Snell (*10*) states that iron interferes with the Dienert-Wandenbulke method. The effects of both ferrous and ferric ions on the suggested method are reported in Table IV. Ferric iron gives no interference throughout the concentration investigated, and the effect of the ferrous iron is negligible in low silica content and not serious in high silica concentration. Average natural and boiler waters contain far less concentration of these ions than is required to give any silica interference.

The temperature of sample and reagents is a factor in this method and should be maintained within ±5° F. of the solution temperature at time of standardization. Temperatures a few degrees below or above this limit give no serious error. High temperatures promote color development with attendant high silica results, while lower temperatures have the opposite effect.

Table IV. Effect of Ferrous and Ferric Ions

Silica as SiO_2			Fe^{+++}	Fe^{++}
Present	Found	Difference	Present	Present
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
6.4	6.3	−0.1	..	..
	6.4	0.0	0.7	..
	6.5	+0.1	1.3	..
	6.3	−0.1	2.7	..
	6.5	+0.1	6.7	..
	6.3	−0.1	13.4	..
	6.3	−0.1	26.8	..
25.5	25.2	−0.3	..	..
	25.3	−0.2	6.7	..
	25.2	−0.3	13.4	..
	25.2	−0.3	26.8	..
6.4	6.3	−0.1	..	0.7
	6.3	−0.1	..	3.4
	6.3	−0.1	..	6.7
	7.6	+1.2	..	13.4
25.5	25.3	−0.2	..	0.4
	25.2	−0.3	..	0.7
	25.0	−0.5	..	1.7
	23.7	−1.8	..	3.3
	19.2	−6.3	..	13.4

Table V. Effect of Alkalinity

Silica as SiO_2			Alkalinity to Phenolphthalein	Alkalinity to Methyl Orange
Present	Found	Difference	*P. p. m. as $CaCO_3$*	
P. p. m.	*P. p. m.*	*P. p. m.*		
5.1	5.2	+0.1	25	55
	5.0	−0.1	240	440
	4.7	−0.4	520	980
25.5	25.6	+0.1	30	60
	25.7	+0.2	170	330
	25.4	−0.1	320	550
	22.5	−3.0	620	1120
36.7	36.5	−0.2	50	75
	36.9	+0.2	152	280
	35.5	−1.2	275	450
	34.7	−2.0	340	550
	31.6	−5.1	675	1195

Table VI. Stability of Yellow Silicomolybdate Complex

Silica as SiO_2 Present	Silica as SiO_2 Found	Silica as SiO_2 Difference	Time Silicomolybdate Complex Stood before Sulfite Added
P. p. m.	*P. p. m.*	*P. p. m.*	*Min.*
5.1	5.0	−0.1	1
	5.0	−0.1	5
	5.2	+0.1	8
	5.2	+0.1	15
	5.2	+0.1	30
25.5	19.2	−6.3	Sulfite added immediately after molybdate
	25.2	−0.3	1
	25.3	−0.2	0.5
	25.2	−0.3	3
	25.2	−0.3	5
	25.3	−0.2	10
	24.5	−1.0	28
45.0	45.3	+0.3	1
	44.5	−0.5	4
	43.5	−1.5	8
	40.8	−4.2	15

Table VII. Color Progression of Molybdenum Blue Color

Silica as SiO_2	Silica Found—Regular 1-minute reading	Silica Found—2-minute reading	Error Caused by Missing Time of Reading—By 1 minute	Error Caused by Missing Time of Reading—By 3 seconds (calculated)
P. p. m.	*P. p. m. SiO_2*		*P. p. m. SiO_2*	*P. p. m. SiO_2*
37.0	36.7	38.1	+1.1	0.06
18.5	18.5	19.4	+0.9	0.045
7.4	7.2	7.7	+0.3	0.015
1.8	1.7	1.9	+0.1	0.005

The effect of alkalinity is given in Table V, which shows no interference in low silica concentration. In high silica concentration total alkalinity can be tolerated to 350 p. p. m. Even though many boiler waters exceed this alkalinity, it is not considered a disadvantage to the method, as suitable dilution of sample reduces alkalinity to a level where it offers no effect. Dilution is often necessary with boiler samples, as their silica contents are beyond the range of the method. Dilution does not introduce appreciable error, as the data in Table VIII show.

Color progression of both the yellow silicomolybdate and the molybdenum blue is of little significance. Approximately a minute is required for the yellow color to attain its maximum development, after which it is completely stable for 5 to 30 minutes, depending on its concentration, as indicated in Table VI. This completely stable time of 5 minutes is more than ample for the complete silica test, so that any change in the yellow silicomolybdate color beyond 5 minutes is of no importance. Table VII shows that the color progression of the blue reduction color is easily controlled. The results as shown are not seriously in error, even if the time is exceeded by 1 minute. Time errors are in the order of 3 seconds and cause no error in the application of this method.

Results on Natural and Boiler Waters

The new procedure was tested on eight actual samples from various parts of the country. The comparison of gravimetric with photometric results is presented in Table VIII and shows that the procedure gives acceptable accuracy.

In the boiler samples, dilution was employed in the photometric method without sacrifice of accuracy. Table IX gives the analysis of the samples and indicates that sulfates, chlorides, and hardness, in addition to the other ions investigated, do not interfere.

Advantages of Photometric Method

This photometric method gives accuracy comparable to gravimetric analysis and requires only a 10-ml. sample and less than 5 minutes for testing. It is sensitive enough to detect 0.1 p. p. m. as silica. The average time required for the gravimetric analysis in this work was approximately 5 hours per duplicate sample, and large volumes of sample were necessary to attain this accuracy. For work involving lower silica concentration below 50 p. p. m. of silica, this method can be modified simply by use of a larger cell depth, and under these conditions silica can be determined more accurately than with the test tube.

Conclusions

The proposed method measures the blue color resulting from the reduction of silicomolybdate complex and was found to give acceptable results compared to gravimetric analysis. The method can tolerate as high as 150 p. p. m. of phosphate as PO_4 and 350 p. p. m. of alkalinity as calcium carbonate without dilution. Tannins show no practical interference.

Table VIII. Gravimetric vs. Photometric Results

Sample	Location	Sample	Silica—Gravimetric	Silica—Photometric	Sample Dilution, Photometric Procedure
		Ml.	*P. p. m. SiO_2*		
Raw	Fitchburg, Mass.	1500	3.1	3.1	..
			3.1	3.2	..
Boiler	Berlin, N. H.	250	70.0	67.8	1:1
			66.8	68.5	1:4
				67.0	1:4
Boiler	Rocky Mount, N. C.	500	65.2	65.4	1:1
			65.8	65.0	1:1
Feed	Rocky Mount, N. C.	1500	1.9	1.6	..
			1.4	1.7	..
Boiler	Philadelphia, Penna.	500	33.4	33.5	..
			34.2	33.8	..
			33.8		
Raw	Philadelphia, Penna.[a]	1000	12.1	12.8	..
			12.6	12.5	..
Boiler	Curwensville, Penna.	75	48.0	46.8	..
				47.2	1:1
Feed	Berlin, N. H.	1500	3.1	3.1	..
			3.2	3.0	..

[a] Fortified with sodium silicate.

Table IX. Analysis of Samples

Sample	SO_4	Cl	Alkalinity[a]—Hardness[a]	Alkalinity[a]—Phenolphthalein	Alkalinity[a]—Methyl orange	PO_4	Total Solids	Organic	Fe	pH
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	..	..	..	..	..	..	..	...	..	..
2	96	20	0	208	400	200	..	300	..	11.3
3	560	48	0	104	176	70	1010	50	..	10.7
4	8	1	4	0	12	..	..	...	0.5	7.1
5	608	88	0	176	304	45	1714	190	..	11.5
6	20	4	48	4	40	..	..	...	..	8.5
7	60	50	..	120	240	..	1460	240	..	10.1
8	8	0.5	18	0	12	..	..	...	..	7.2

[a] As $CaCO_3$.

Ferric and ferrous iron, chlorides, sulfates, and mineral hardness offer no interference. The Dienert-Wandenbulke method is not considered suitable for accurate silica determination in waters containing tannins, which interfere seriously by forming yellow tannin-molybdate complexes.

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Anhydrous Sodium Thiosulfate, A Primary Standard

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THE reaction between bromoacetate and thiosulfate ions has been found by Slator (*8*) and by La Mer and his coworkers (*4*, *5*, *6*, *9*) to be uniquely free of side reactions. Accordingly, the measurement of velocity constants using these reactants offers a critical method of detecting the existence of decomposition products of either reagent. By employing such kinetic measurements, Kamner and Tomlinson working with La Mer (*6*, *7*) have shown that the pentahydrate of sodium thiosulfate decomposes upon standing at room temperature for several months after recrystallization. At the suggestion of La Mer, the investigation has been extended to include the stability of the anhydrous salt.

In 1904, Young (*10*) suggested the use of the anhydrous salt as a primary standard but presented only meager supporting data. This paper shows that anhydrous sodium thiosulfate is stable thermally at 120° C. and meets the requirements of a primary standard so well that it deserves more extended use.

The methods of purification of reagents and the technique of reaction velocity measurements were those of La Mer and Kamner. Water solutions 0.01 *M* in reactants were employed. After two kinetic determinations, sodium thiosulfate pentahydrate was placed in an oven at 120° C. Portions of the dehydrated product were used for subsequent reaction rate measurements. The data in Table I show conclusively that this thiosulfate was not decomposed by the heat to which it was subjected over a period of 11 weeks. The drifts in velocity constants noted by La Mer and Tomlinson using the pentahydrate which had been kept at room temperature for 2 years were not observed.

Samples of C. P. sodium thiosulfate pentahydrate were dried at 120° C. to constant mass and stored over calcium chloride. Portions of these samples were recrystallized from water below 65° and then subjected to dehydration to ascertain the importance of recrystallization. The time required for dehydration depends, of course, upon the frequency of stirring the solution formed upon heating the hydrated salt and later upon the frequency and degree of subdivision. Individual samples of the thiosulfate (0.2 to 0.4 gram) were titrated with 40 to 50 ml. of standard iodine solutions using calibrated volumetric apparatus; the iodine purification, solution preparation, and titrations were carried out after the method of Kamner (*3*). C. P. resublimed iodine was sublimed from a 1 to 5 potassium iodide mixture and this product was sublimed again, dried, and stored over calcium chloride. All iodine solutions contained 4 per cent of potassium iodide. Freshly prepared solutions of soluble starch (0.5 per cent) were used and correction blanks determined.

From Table II, it may be seen that the concentrations of iodine solutions determined against weighed portions of these anhydrous samples and the values calculated from the masses of iodine used are in good agreement, even when the C. P. grade salts were employed without recrystallization. The analysis of the dehydrated, unrecrystallized sample 2 indicated that it was slightly decomposed. This was confirmed by kinetic measurements upon both dehydrated and original samples. The velocity constants so obtained exhibited pronounced drifts which corresponded to those observed in previous investigations.

When an accuracy greater than 2 parts per thousand is not obligatory, the data indicate that the C. P. salt of commerce may not require recrystallization. Upon recrystallization, the results are in agreement well within the limit of experimental error.

Arsenious oxide (99.82 per cent As_2O_3 *vs.* Bureau of Standards Sample 83) was used to determine (*1*) independently the normalities of two standard iodine solutions prepared by weight. The excellent agreement between these iodine concentrations determined by arsenite and thiosulfate titrations is shown in Table III. Moreover, it is evident from the precision obtained that no marked change had occurred in the composition of these anhydrous samples which had been stored in a bright laboratory, but seldom in direct sunlight,

TABLE I. EFFECT OF HEATING SODIUM THIOSULFATE

(As determined by rate of reaction with sodium bromoacetate)

Days Kept at 120° C.	Velocity Constant, k
0	0.489 ± 0.001
0	0.490 ± 0.000
2	0.490 ± 0.002
4	0.487 ± 0.002
9	0.492 ± 0.002
16	0.492 ± 0.001
28	0.490 ± 0.001
79	0.488 ± 0.001

$k_{av.} = 0.490 \pm 0.001$

TABLE II. ANALYSES OF IODINE SOLUTIONS BY SODIUM THIOSULFATE

Sample	Normality by $Na_2S_2O_3$	Normality Calcd. from I_2 Content	Deviation %
1	0.05825	0.05828	0.05
1 recrystallized	0.04045	0.04046	0.03
2	0.05825	0.05839	0.24
2 recrystallized	0.04045	0.04045	0.00
3	0.05825	0.05832	0.12
3 recrystallized	0.05825	0.05825	0.00
4	0.05825	0.05822	0.05
4 recrystallized	0.05590[a]	0.05592	0.04
	0.05825[b]	0.05825	0.00
	0.04255[c]	0.04257	0.05

Sample at 120° for [a] 18 days, [b] 28 days, [c] 79 days.

Table III. Analyses of Iodine Solutions by Sodium Thiosulfate and by Arsenious Oxide

$Na_2S_2O_3$ Sample No.	Normality by $Na_2S_2O_3$	Normality by As_2O_3	Normality Calcd. from I_2 Content
1 recrystallized	0.04256	0.04253	0.04255
2 recrystallized	0.04323	0.04319	0.04320
3 recrystallized	0.04254	0.04253	0.04255
4 recrystallized	0.04322	0.04319	0.04320

Table IV. Analyses of Ceric Sulfate Solutions by Sodium Thiosulfate after Prolonged Exposure to Light

Sample	Normality by $Na_2S_2O_3$	Normality by Mohr's Salt	Deviation %
1 recrystallized	0.04564	0.04562	0.04
2 recrystallized	0.04572	0.04562	0.22
3 recrystallized	0.04565	0.04562	0.07

for a month over calcium chloride. Further substantiation of this stability toward light, in the absence of moisture, is presented in Table IV. These samples were stored similarly for 11 months, then separate portions (0.25 to 0.35 gram) were titrated with ceric sulfate solution (*2*).

In order to determine the possibility of decomposition of anhydrous sodium thiosulfate exposed to both light and air, portions were exposed to the atmosphere of the laboratory in weighing bottles set in beakers covered with watch glasses. After known periods of exposure, the samples were heated at 120° C. to constant mass, and separate portions were titrated with the ceric sulfate previously employed.

Typical resulting data appear in Table V. Obviously, detectable decomposition had not occurred.

Finally, sodium thiosulfate was found to be not appreciably hygroscopic and readily redriable to constant mass. A portion of sample 5 which had taken on 1.6 per cent of its weight of water during 7 days' exposure to the air was dried to its original mass in 2 hours at 120°. A portion of sample 1 with similar surface exposed showed no change in mass over a period of 6 days under similar conditions. A 4.503-gram portion of sample 2 gained 48 mg. in 18 days but was redried completely in 1 hour at 120°.

Summary

The stability of anhydrous sodium thiosulfate has been investigated. No decomposition has been indicated by the critical method of reaction velocity measurements with sodium bromoacetate when the salt was kept at 120° C. for 79 days. The dehydration of sodium thiosulfate pentahydrate produces an anhydrous salt which fulfills the requirements of a primary standard.

Table V. Effect of Exposure to Light and Air upon Composition of Sodium Thiosulfate

(Normality of $Ce(SO_4)_2$ by Mohr's salt, 0.04562)

Sample	Days Exposed	Normality of $Ce(SO_4)_2$ by $Na_2S_2O_3$	Deviation %
1 recrystallized	18	0.04561	0.02
	24	0.04563	0.02
	32	0.04557	0.11
2 recrystallized	19	0.04559	0.07
	31	0.04562	0.00
3 recrystallized	21	0.04560	0.04
	34	0.04564	0.04
5 recrystallized	0	0.04557	0.11
	23	0.04562	0.00

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Quantitative Spectrochemical Method for Zinc Die Casting Analysis

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THE literature of the last few years pertaining to spectrochemical analysis by means of the emission spectrum indicates clearly that a new era in speed, accuracy, and dependability of this type of analysis has been ushered in. The 10-minute analysis of steel for silicon, manganese, chromium, nickel, and molybdenum, with an inaccuracy of less than 3 per cent of the quantity being measured in all cases, is a typical example (*4, 5, 6*). These gains in speed, accuracy, and dependability have been achieved through no revolutionary innovations but rather through a realization of all the factors making for a good analysis. Apparatus designed with these factors in mind is now available, so that any laboratory can be assured of definite results in connection with the analysis of certain materials.

The extension of these contemporary methods to the analysis of a wide variety of materials is the immediate need in spectrochemistry. The development of a new modification of the standard direct current arc (*1*) by these laboratories has made possible rapid and accurate analyses of materials that have been analyzed heretofore by tedious spectrographic or chemical methods. The present paper describes the application of this source and a standard group of instruments to the analysis of one such type of material, zinc die casting alloys.

Since 1935 the American Society for Testing Materials has recommended a spectrochemical method (*2*) suitable for the analysis of lead, tin, cadmium, magnesium, iron, and copper in both zinc and zinc alloys such as are used in die casting. The method has enjoyed considerable popularity due to its inherent advantages when compared with routine chemical methods.

These advantages are, in the main, connected with the spectrograph's ability to register lines of the various elements just as accurately at very low concentrational levels as at the higher levels. In other words, the spectrograph allows a constant degree of accuracy nearly independent of the quantity of an element present. For this reason errors in this analytical method are always referred to as a certain percentage of the quantity present, be that quantity 0.001 or 10 per cent. Many routine chemical methods, on the other hand, have a fixed absolute error expressed as a certain percentage of the entire sample and not dependent upon the amount being

measured. When this amount approaches the absolute error, very large variations in answers will be obtained by different analysts and even the most accurate methods fail.

In producing die castings it is imperative that lead, tin, and cadmium be determined down to concentrations of 0.001 per cent, as little more than this can be tolerated in the better castings, and other constituents like magnesium, iron, and copper must often be held to lower levels of a few hundredths per cent. Thus, by and large the problem appears ill suited for analytical chemistry and rather ideally suited for spectrochemistry. Experience has borne out this conclusion time and again, always giving impetus to the greater use of spectrochemical methods.

Of the various spectrochemical methods available, the A. S. T. M. method has a simplicity and straightforwardness that has made it the model for many analyses of this type. In general, however, the method does not lend itself to the latest trends in spectrochemistry: (1) to eliminate the use of standards except for the original calibration and occasional check of the analytical system; (2) to eliminate all complicated sample preparation; and (3) to increase the accuracy of analyses over those previously available. The method described here, on the other hand, lends itself admirably to the very latest practices, thus ensuring speed, certainty, and a high degree of accuracy. Two ways of handling the original samples are described.

Metal Peg Analysis

To achieve 2 man-minute analyses sample preparation must be reduced to a minimum. Working with the metal directly is the most rapid. In foundry work where the molten metal is available, sample rods may be cast to the required dimensions. In testing fabricated stock, small rods can usually be machined in a very short time. The test rods utilized are 0.23 cm. (0.094 inch) in diameter and 2.2 cm. (0.875 inch) long, and fit directly into a special base illustrated in Figure 1. This base also holds an insulating Transite bushing, over which is placed a graphite sleeve. Ammonium chloride is used to fill the space between the rod and the sleeve. The analytical reagent grade, produced in a finely powdered form, works very well without any grinding or preparation, except that it must be well dried before using. In practice it is kept in a desiccator over sulfuric acid. The Transite bushings and the graphite sleeves may both be used over and over again, the former being cleaned mechanically, the latter by nitric acid treatment.

The arc is completed with the upper electrode shown, produced by pushing a small length of 0.3-cm. (0.125-inch) carbon electrode into a 0.6-cm. (0.25-inch) carbon, drilled and slotted to receive the smaller size. The arc is operated with the electrodes 0.9 cm. (0.375 inch) apart and must be exactly aligned with the spectrograph. The arc-spark stand utilized allows the projection of images of the electrodes onto a marked ground-glass screen which provides exact spacing and alignment. The arc is struck by lowering the top electrode with a knob mechanism, and is then spaced by turning this knob until a stop is reached. Because of the very short arcing times employed, 4 seconds, it is necessary that the arc may be accurately spaced in a few tenths of a second. The 4-second interval is controlled by an electric timer on the rectifier which extinguishes the arc after operating the preset time. Ten amperes are passed through the arc circuit, this value being attained just as the arc is opened to its full spacing. To insure reproducibility of this value the standard rectifier is adjusted before arcing to supply 14 amperes when carbon electrodes are short-circuited in the arc-spark stand.

This type of arc blows a large volume of sample through the discharge by means of the gas formed by decomposing ammonium chloride, and because of this action has been termed a "high streaming velocity arc". In the 4 seconds of arcing about 75 mg. of sample are forced up through the discharge. This makes for high atomic concentrations and great spectral intensity, so that a single 4-second exposure is adequate to produce an intense photographed spectrum with the high-resolution grating spectrograph employed.

This same type of arc has been tested on a variety of metals and found to give very promising results. An iron peg of the same dimensions as the sample peg gives a highly reproducible arc when operated with ammonium chloride and 7.5 amperes in the arc circuit. For this value the rectifier is adjusted to 10.6 amperes on short circuit. Lengths of finishing nails of the correct diameter furnish the iron rods, which are held in the same type of holder as the sample rods. Extended tests have shown that the relative intensities of the various iron lines as produced in this arc are reproducible to a few per cent, which is much better than was obtainable with any other iron arc tried, including that reported by Slavin (*3*).

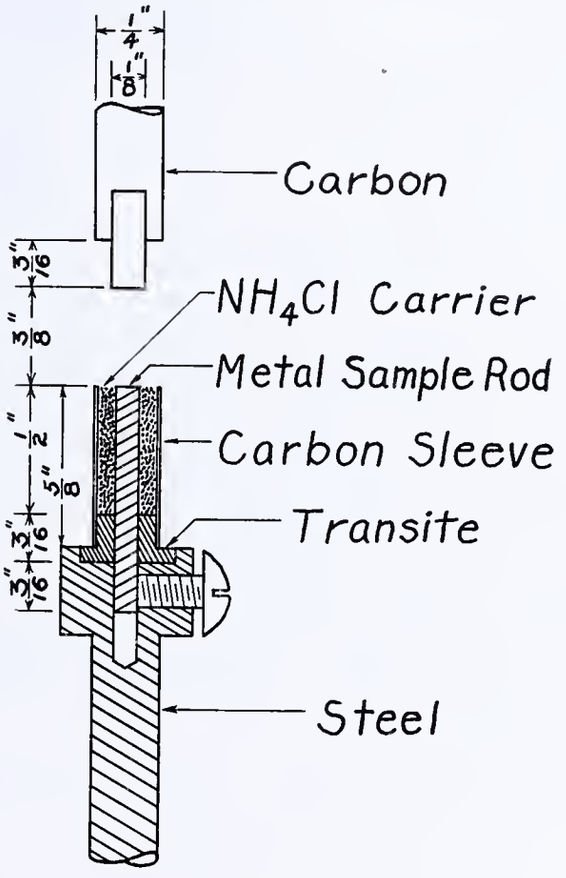

FIGURE 1

The constancy of the relative intensities in this arc makes it entirely suitable for film calibration, as to both emulsion contrast and sensitivity in the various regions of the spectrum. At the same time it is used for line identification in connection with a standard dual projection comparator. Thus two sources are available, one for sample analysis and one for film calibration.

The spectrograph used to photograph the spectra is a standard A. R. L. grating spectrograph with good dispersion and resolution. Its dispersion, 7 Å. per mm., is adequate to give good line separations with the 60-micron slit used for accurate densitometry even with considerable iron in the samples. This also allows accurate measurement of background next to the line, which is very important when making quantitative determinations close to the limit of detection. Another point in connection with background measurements is that not much scattered light can be tolerated in the spectrograph. The superiority of the concave grating spectrograph over a prism instrument in this respect is really worth while whenever very small concentrations must be measured with high accuracy. The standard ultraviolet range of the spectrograph, 2380 to 4600 Å., was used for all the quantitative determinations involved in zinc die casting material analysis. However, a special range, 2280 to 4500 Å., which includes the 2288 cadmium line and all other lines needed, was also available and could be used regularly just as well as the standard range if higher sensitivity for cadmium analysis were essential.

The optical system between the spectrograph and the source is simple and convenient. A rotating sector and aperture, placed at the secondary source plane of the astigmatic grating spectrograph, controls the speed of the instrument and defines the spec-

Table I. Typical Analytical Data

Iron Calibration Spectrum

Calibration group										
1. Wave length, Å.	2788.1	2786.8	2786.2	2781.8	2778.8	2778.2	2767.5			
Known relative intensity	1.92	0.219	0.126	0.619	0.519	1.18	1.00			
Percentage transmission[a]	8.8	72	88	35.7	42.0	15.1	19.5			
2. Wave length, Å.	3173.7	3172.1	3171.4	3168.9	3166.4	3165.9	3164.3	3163.9	3163.1	
Known relative intensity	0.97	0.64	1.00	0.65	1.95	1.32	0.35	0.42	0.22	
Percentage transmission	37.4	52.3	35.7	52.0	18.0	27.1	75	68.8	88	
3. Wave length, Å.	3953.8	3948.8	3948.1	3947.5	3947.0	3943.4	3942.4	3940.9	3940.0	3852.4
Known relative intensity	0.136	1.20	0.86	0.57	0.44	0.24	0.77	0.80	0.066	1.00
Percentage transmission	65.0	6.8	10.3	16.8	23.0	43.0	11.8	11.2	87	8.6

Die Casting Alloy Spectrum Example

Element	Zinc	Magnesium	Copper	Aluminum	Tin	Cadium	Iron	Lead
Wave length of line, Å.	2670	2783	2961	3064	3175	3261	3737	4058
Transmission of line and background	11.2	14.7	13.3	24.1	89	61.2	16.6	80.3
Transmission of background	90	66	84	78	95	89	99	99
Calibration group used	1	1	1	2	2	2	3	3
Intensity of line and background	1.57	1.24	1.36	1.48	0.21	0.52	0.58	0.087
Intensity of background	0.11	0.25	0.15	0.32	0.15	0.21	0.029	0.029
Intensity of line	1.46	0.99	1.21	1.16	0.06	0.31	0.55	0.058
Intensity ratio to Zn line		0.68	0.83	0.79	0.04	0.21	0.38	0.040
Multiplicative constant, α	...	0.052	1.38	4.78	0.0252	0.0131	0.0608	0.069
% of element present	...	0.035	1.14	3.77	0.001	0.0027	0.023	0.0028

[a] Percentage transmission is referred to clear film transmission, which is considered as 100%.

tra vertically. This same sector carries on its periphery a stepped sector which may be used for density calibration if desired. The source is 25 cm. beyond this aperture, no lenses being employed between it and the spectrograph unless the stepped sector is used.

The camera of the spectrograph takes a 3000-cm. (100-foot) roll of 35-mm. motion picture film. A 31.6-cm. (12.625-inch) length is used to photograph spectra of eight samples and one standard iron. This length is removed through an aperture at the side of the camera. The spectrograph is then ready for the next set of analyses. Eastman Par Speed film is used to ensure approximate equality of contrast throughout the spectral range photographed. An Inconel film holder and a developing machine allow convenient film processing. To ensure uniform development over each portion of the film a modified brush development technique is used, employing a rubber blade on a paddle which is moved along the length of the film about 0.16 cm. (0.06 inch) above the emulsion. This is spaced from the film accurately by the handle of the film holder.

The D-72 stock developer used provides adequate development in approximately 1 minute. A few seconds in the short stop arrests development. Fixing is achieved in 1 minute, washing in 0.5 minute, and drying takes another 3 minutes. Thus, the total film processing time is about 6 minutes.

The film, when dried, is placed in a standard dual projection comparator. By alignment between the iron spectrum on the film and one on the master, the wave length of any line in the spectrum can be read directly from a scale on the master. Besides this, most of the lines used in the analysis are shown and labeled on the master plate directly, so that line identification is very rapid and certain. Transmission readings on these lines are obtained with a standard photoelectric densitometer. By utilizing a mechanical sweep on the densitometer and slit mechanism, this instrument allows readings accurate to a few tenths of a per cent to be made very rapidly. The actual readings used are on the lines shown in Table I.

The relative intensities of each group of iron lines were determined by standard stepped-sector methods. Once these were obtained, however, they were used for film calibration at all times. Successive lines appearing in the iron spectrum in one region of the spectrum make a calibrating set for that region. By arbitrarily calling one line unit intensity, all readings taken in that region can be converted to intensities with respect to this unity line. This can be done in each region of the spectrum separately, irrespective of the changing film contrast or sensitivity from one region to the other. Comparison between intensities of lines in different regions can also be made, since the unity lines in the different regions of the spectrum maintain their relative intensities constant with this type of iron arc. Thus, instead of measuring, for instance, $\frac{I(\text{Pb4058})}{I(\text{Zn2670})}$ directly, which is impossible photographically because of the indeterminate film sensitivities in the two regions, $\frac{I(\text{Pb4058})}{I(\text{Fe3852})}\frac{I(\text{Fe2767})}{I(\text{Zn2670})}$ is measured, which is possible photographically. Since $I(\text{Fe2767})/I(\text{Fe3852})$ is a constant it can be absorbed into the other calibrating constants of the system. Thus, this method of calibration allows the intensity comparison of any two lines appearing in the photographed spectrum, irrespective of their difference in wave length. This allows the use of a single zinc line 2670 as an internal standard for all element lines, irrespective of the region of the spectrum in which they appear.

It may be seen from Table I that the spectrum has been divided into three regions, each being calibrated and used for certain determinations. The actual conversion of line transmission readings to intensity values is done on a calculator.

The principle of operation is simple. A plot of the data using log transmission as ordinates and log intensities as abscissas gives three film calibration curves. The first group of lines of the alloy spectrum would be read from curve one, the 2800 Å. region calibration, the second from curve 2, etc., giving the intensities. Besides the intensity of the line, which really represents line plus background, the background intensity can be determined from a transmission reading next to the line. Then by subtracting this intensity value from that of the line plus background, a true line intensity is obtained. This is illustrated in the example.

It is obvious from the size of this correction that it cannot be ignored. In fact, it is only by making such a correction that accurate results can be obtained at very small concentrations when a line superimposed on the background may have only a fraction of the background intensity. From these corrected intensities the ratios of intensities are determined with reference to the internal standard zinc line. The ratios for lines in groups 2 and 3 have an arbitrary factor included in this ratio, but since this is a constant it introduces no complications into the system.

With the intensity ratios available the final conversion to percentage can be made. This requires that these ratios be obtained from runs on a number of samples of known composition, so that the relationship between intensity ratio and percentage may be established. A method of obtaining accurate analyses of standards is given below. For all the elements in question, over a certain range of percentages, a direct proportionality exists between intensity ratio and percentage $C = \alpha I$ (element line)$/I$(standard line). Thus, by determining α for each element the complete analytical system is determined. These constants are given in Table II, together with the range of percentages in which they can be used. From them the percentages of the example shown in Table I were obtained. The fact that a single constant can be used to define the analysis of each element is very convenient and represents one of the main advantages of this type of source when coupled with this manner of calculation.

This procedure may appear involved, but it serves for the calculation of the analyses of eight samples for seven elements each or fifty-six individual element analyses. A special calculator including various rules, which allows the final percentages to be obtained directly from the transmission values, was used in the actual tests and calibration of the system. This calculator will be described in detail at a later date.

For lead, tin, and cadmium the lower percentage figure of Table II represents the lowest limit that can be measured with an accuracy of 0.0001 per cent. All the other limits can be extended to lower or higher percentages as required. The accuracy obtainable in the analysis of the various elements has been studied in some detail. When eight pegs cut from a pig are used to obtain eight repeat analyses, assuming that no segregation exists, the inherent errors of the spectrographic method are easily calculated. This has been done for each element and at a number of different concentrations when samples were available. The results have been very gratifying, indicating a new degree of accuracy in arc-spectrum analysis. Aluminum at 4 per cent can be analyzed with an average error of 0.1 per cent, if good nonoxidized metal pegs are available. Copper determinations show an average error of 2 to 3 per cent of the quantity being measured, or about 0.03 per cent at 1 per cent and 0.09 per cent at 3 per cent. Magnesium and iron values have an average error of from 4 to 6 per cent of the quantity being measured, 0.0006 per cent at 0.01 per cent and 0.004 per cent at 0.1 per cent. The accuracy of determinations at the lower limit of detection of lead, tin, and cadmium has been indicated above, while the average error at 0.01 per cent is approximately 5 per cent of that quantity or 0.0005 per cent.

A set of eight analyses requires 18 minutes to set up and arc the sample pegs, 6 minutes to process the film, 30 minutes to obtain the transmission readings, and 30 minutes' calculating time, or a total of 84 minutes to obtain 56 individual analyses. This represents an average time of less than 2 man-minutes an analysis if sample rods are available.

Thus, a metal rod analytical system has been devised for die casting alloys which is comparable in speed and facility to the spark method of iron analysis. The fact that it furnishes accurate analyses in both the 1 to 4 per cent range and the 0.001 to 0.01 per cent simultaneously shows that the source used is a valuable addition to the present types of spectral sources available.

Table II. Calibrating Data for Metal Peg Analysis

(Using 2670 zinc line as an internal standard)

Element	Wave Length of Line	Range Covered	Multiplicative Factor, α	Calibration Group
	Å.	%		
Magnesium	2783	0.01–0.1	0.052	1
Copper	2961	0.05–1.0	1.38	1
Aluminum	3064	3.5–4.5	4.78	2
Tin	3175	0.0009–0.01	0.0252	2
Copper	3194	0.3–3.0	3.00	2
Cadmium	3261	0.0009–0.01	0.0131	2
Iron	3737	0.01–0.1	0.0608	3
Lead	4058	0.0006–0.01	0.069	3

Metal Oxide Analysis

There are certain cases where it is either not possible or advantageous to obtain a rod for analysis. In the case of custom work, drillings are often submitted for analysis, while when large samples must be tested, drillings from various portions are necessary to furnish a truly representative sample. In that case acid solution must be used to furnish the smaller representative sample used in an arcing. It was to work with this type of sample that the A. S. T. M. (*2*) method was devised.

It is much more important to have a nonmetallic sample method available for analysis of the standards used to calibrate the metal peg arcing method. These could be analyzed by another method—for instance, chemical analysis—but any errors inherent in that analysis would be reflected in the final system. However, with the nonmetallic sample method available any metal sample can be reduced to a nonmetallic and compared directly with synthetically prepared nonmetallics produced by the addition of known amounts of all the constituents. Once the analysis of certain samples is thus determined, metal pegs of this material can serve as standards for the direct metal peg analysis.

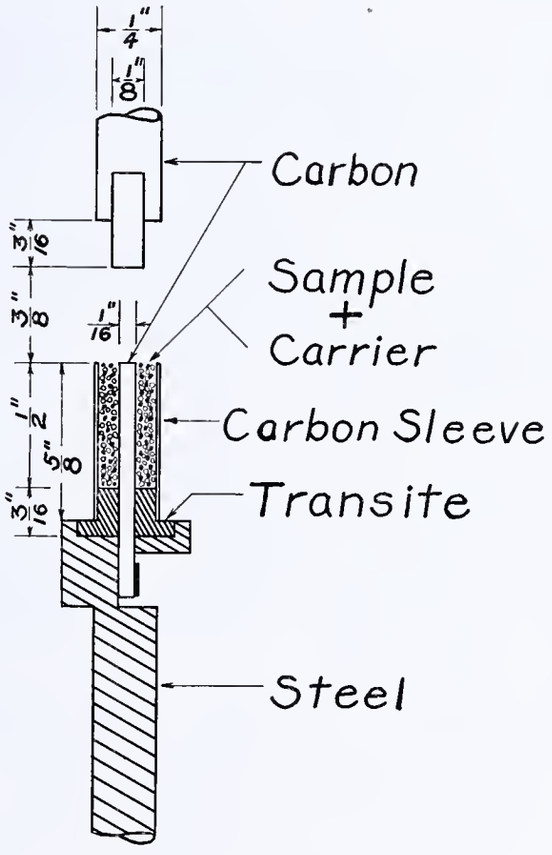

Figure 2

The method devised utilizes powdered oxides prepared from solution of the sample in acid. The procedure given below was devised after many tests and represents the one that gave the most reproducible results day after day. It has been checked for possible loss of any constituents and found satisfactory in this respect. It should be followed closely to assure the production of oxides in a manner that can be reproduced from sample to sample and from time to time.

A mixture of 5 cc. of water, 5 cc. of concentrated nitric acid, and 0.5 cc. of concentrated hydrochloric acid per gram of metal is used to dissolve the sample. Water and acid are mixed in the order given just before adding them to the metal. The sample plus acid is heated until all the metal appears to be in solution and then let stand hot for 5 minutes. The final oxides are formed by pipetting this solution drop by drop into a silica dish with a flat bottom, heated to about 350° C. Drops should be placed all over the bottom of the dish, each one forming oxides in turn. This allows each drop to go through the liquid, solid nitrate, solid oxide transition as an individual portion. Only by such treatment is it possible to secure oxides that give reproducible results no matter when produced. Larger volumes of solutions heated to produce oxides gave erratic results, probably because of the possibility of forming a variety of compounds of zinc, aluminum, and oxygen, depending upon the exact heating cycle.

An analogous effect probably occurs when electrodes are prepared with 0.1 cc. of acid solution of the sample in the A. S. T. M. method, as it is impossible to duplicate intensity ratios from day to day with that method. It is probably for this reason that

TABLE III. CALIBRATING DATA FOR OXIDE ANALYSIS

(Using 2670 zinc line as an internal standard)

Element	Wave Length of Line Å.	Range Covered %	Multiplicative Factor, α	Calibration Group
Magnesium	2783	0.01–0.1	0.088	1
Iron	2813	0.01–0.05	0.11	1
Copper	2961	0.05–0.3	0.286	1
Aluminum	3064	3.5–4.5	4.12	2
Tin	3175	0.0002–0.01	0.0028	2
Copper	3194	0.3–3.0	0.76	2
Cadmium	3261	0.0007–0.01	0.0082	2
Lead	3683	0.0003–0.01	0.0091	3
Iron	4271.7	0.01–0.1	0.052	3

standards must always be prepared simultaneously with samples, so that they will all pass through the same heating and drying cycle. Accurate analyses can, of course, thus be attained, though at the price of always repeating the preparation of standards for each new batch of samples.

After an oxide has been prepared in the silica dish, it is removed by gentle scraping and ground in an agate mortar. It is then mixed with carbon as a carrier which is used to blow the oxide up through the arc. Sucrose that has been carbonized and ground in an agate mortar to <200-mesh produces carbon that reacts readily with the atmosphere to produce large volumes of carbon monoxide and carbon dioxide. A one to one mixture by weight of sample and carrier is used. Ammonium chloride could not be used, since it reacted with the zinc to form a compound of low melting point.

The carbon mixture is arced in an electrode such as is depicted in Figure 2. A 0.16-cm. (0.06-inch) carbon rod at the center carries the current, while a 0.6-cm. (0.25-inch) carbon sleeve 1.6 cm. (0.625-inch) long, insulated from the electrode with a Transite bushing, acts as the sample-holding chamber. To protect the Transite bushing from the intense heat produced, a layer of carbon is placed over it. Electrode carbon filings thermally insulate and yet do not tend to act as a carrier. The remainder of the space is filled with the reactive carbon-sample mixture, about 250 mg. being required. Light tapping allows complete filling.

The same spacing and alignment of electrodes as employed with the metal pegs are used. The current and timing are also the same. In preparing the set of standard oxides from which the metal oxide working curves were determined once and for all, standard solutions were used for most of the elements. These were neutral solutions of aluminum nitrate, 40 mg. of aluminum per cc.; magnesium nitrate, 1 mg. of magnesium per cc.; ferric nitrate, 1 mg. of iron per cc.; lead nitrate, 0.1 mg. of lead per cc.; cadmium nitrate, 0.1 mg. of cadmium per cc.; and a tin tartrate solution 0.1 mg. of tin per cc. in 0.2 *N* hydrochloric acid. The tin solution should be prepared just before using.

Take zinc metal of high purity and place 930 mg. in a dish. Then add 1 cc. of aluminum nitrate, 40 mg. of aluminum per cc. to the zinc, and 30 mg. of pure copper wire. Prepare a solution of each of the other solutions, 0.8 cc. in this case, and make up to 4 cc. with water when necessary. Add 5 cc. of nitric acid and 0.5 cc. of hydrochloric acid to this solution. Pour this over the prepared zinc, aluminum, and copper. Proceed just as in the case of a whole metal sample once the zinc has dissolved. This makes a standard oxide with 4 per cent aluminum, 3 per cent copper, 0.08 per cent magnesium and iron, and 0.008 per cent cadmium, lead, and tin.

Prepare two other standards of 1 and 2 per cent copper, 0.02 and 0.04 per cent magnesium and iron, 0.002 and 0.004 per cent cadmium, lead, and tin, but with 4 per cent aluminum in each case.

Careful tests showed that varying percentages of all test elements with the exception of aluminum had no effect on the analysis of each other. Varying percentages of aluminum do have an effect, but fortunately this element is controlled accurately in commercial alloys. Variations from 3.5 to 4.5 per cent did not affect the analyses of the other elements appreciably but did provide a linear change with percentage in aluminum line intensities as referred to zinc, that made possible the analysis of this element over this 1 per cent range.

Because of the effect of aluminum, separate standards must be prepared for alloys without aluminum. A detailed presentation of this effect will be given elsewhere.

All the other spectrographic procedure is exactly the same as for the metal pegs, except that some other spectrum lines are used and where the same line is used a different intensity is obtained, owing to the different chemical form of the sample. For convenience these data are presented in Table III. Again, just as in the metal peg case, simple multiplicative factors may be used to convert intensity ratios to percentage.

With such a method of sample oxide analysis available, calibration can proceed with the regular peg analysis. Duplicate sample pegs can be cut from the material to be analyzed, as indicated in Figure 3. Ends *A* are used for the metal peg arcing to

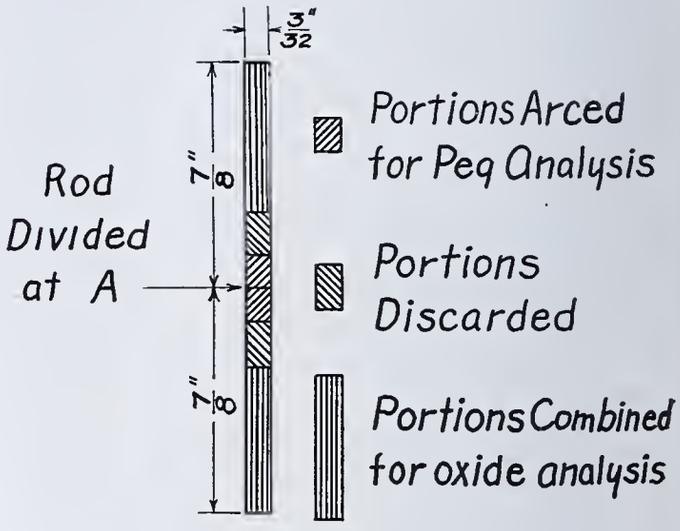

FIGURE 3

produce duplicate spectrograms. The oxidized portions are sawed off and the rest of the two pegs, about 1 gram, is combined and put into solution as indicated above. The oxide is then formed and analyzed by the powder procedure. This analysis, together with the intensity ratios obtained from the direct peg arcing, enables the multiplicative factors for the peg method to be deduced. After these factors have been checked a few times on a variety of samples covering the analytical working range, the system may be considered as calibrated. The factors so obtained should be similar to those given in Table II, as this system of calibration is such that differences in equipment should cancel out to a considerable extent. However, such calibration is essential if the full accuracy of the system is to be realized.

Summary

A complete spectrochemical system has been devised for the analysis of zinc die casting material. A method of calibration is presented, as well as all the analytical factors obtained by the authors. This should allow immediate use of the system by any laboratory equipped with a direct current arc source, a spectrograph of adequate dispersion, and a precision densitometer. The arcing methods described can, of course, be used without a densitometer but with standards to form a modified A. S. T. M. method.

Acknowledgment

The authors would like to express their appreciation to the New Jersey Zinc Co. and F. Porter of the Precision Casting Co. for furnishing a variety of samples suitable for calibration purposes.

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Rapid Preliminary Determination of Melting Points

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THE continuous growth in the application of qualitative organic analysis in industry and the widespread introduction of courses in organic analysis in colleges and universities have brought many developments in laboratory technique, especially in the determination of such frequently employed constants as the melting point.

Considerable time may be consumed in waiting for heat reservoirs to warm to the fusion point of the solid. With an unknown compound, the melting point may be overrun considerably because of too rapid heating of the bath and still more time is required to allow the bath to cool and to prepare a second capillary tube. Beginning students tend to heat the ordinary oil or sulfuric acid baths to too high temperatures, resulting in excessive fuming and destruction of the oil through decomposition. In rarer instances determination of the melting point by capillary tube methods may result in the loss of an appreciable fraction of the derivative, if only a small amount is available. Where a solid is being purified by successive recrystallizations, determination of the melting point by ordinary methods after each crystallization results in considerable loss of time.

The chance observation that the melting point may be determined with minute amounts of solids by the simple method outlined below was considered to merit further investigation because of its surprising accuracy and economy in time and materials. The method is in no way meant to supplant the final and more accurate determinations by the bath–capillary tube techniques where sufficient amounts of solid are available (*1*).

PROCEDURE A, MELTING POINTS BELOW 175° C. Two milligrams or less of the finely ground solid are placed upon the bulb of an ordinary 150° or 360° C. laboratory thermometer having a thin glass mercury reservoir. The position of the solid is such that the temperature may be read by a direct glance at the graduations of the thermometer, no solid being within 0.25 inch of the tip of the mercury bulb. The thermometer is held in a horizontal position with the bulb approximately 8 inches above a 2- or 3-inch nonluminous Bunsen burner flame, the solid resting on the top surface of the bulb. Attention is focused on the thermometer bulb as it is slowly lowered 1 inch every 10 seconds. When the solid fuses at any point on the upper surface of the bulb, the temperature is quickly read without withdrawing the bulb from above the flame. The accuracy of the determination may be increased by spreading the molten solid in a thin film along the bulb by means of a clean spatula or glass rod, if the solid has not decomposed on melting. When allowed to cool and become crystalline, the film becomes clearly visible on the shiny bulb. The heating procedure is repeated and the melting point taken when the film disappears.

TABLE I. RAPID PRELIMINARY DETERMINATION OF MELTING POINTS

Compound	Determination I	II	III	IV	V	Average	Oil Bath	Literature	
Procedure A									
Phenol	42	41	41	42	41	41	40–41	41	
Benzophenone	48	49	48	50	48	49	49–50	49–51	
1,3-Diphenoxy-2-propanol	78	80	83	81	80	80	79.5–80	80–81	
Ethyl-3,5-dinitrobenzoate	92	95	93	94	96	94	92–92.5	93	
Dibenzalacetone	111	113	113	114	112	113	111–112	112	
Benzoic acid	122	122	118	119	118	120	121–122	122	
Cinnamide	146	148	146	147	147	147	145–146	147	
2,4-Dinitrophenylhydrazone of propionaldehyde	157	157	154	158	154	156	154–155	154	
β-Bromoethyl amine hydrobromide	177	172	171	177	173	174	170–171	173	
Procedure B									Procedure A
β-Bromoethyl amine hydrobromide	168	171	170	172	170	170	170–171	173	174
Semicarbazone α-methyl-β-ethylacrolein	190	189	191	193	191	191	187–188	189	194
3,5-Dinitrobenzoic acid	200	205	202	200	202	202	204–205	204–5	209
Phthalimide	228	230	228	232	233	230	231–232	234	236
Phenolphthalein	258	264	257	260	258	259	259–260	261	265
Mixed Melting Points, Procedure A									
Mixture						Range			
Urea + Cinnamic acid	103–8	106–10	106–9	103–10	104–8	103–110	98–110	...	...
Succinic acid + Semicarbazone α-methyl-β-ethylacrolein	142–50	145–53	145–52	148–55	143–49	142–155	146–155	...	...

Results of the determination of the melting points of a number of solids compared with the known melting points as determined in an oil bath are listed in Table I, which includes examples of application to the determination of mixed melting points. The average time required for any single determination is less than a minute. However, in the neighborhood of 175°, the heat exchange is so variable that the individual melting point determinations occasionally vary by over 5°.

PROCEDURE B, MELTING POINTS ABOVE 175° C. The melting point is determined by Procedure A, 10° are subtracted from this temperature, and the solid is again heated, the eye being focused on the scale of the thermometer until melting point $A - 10$ or temperature B is attained. If the solid has not melted, the mean between A and B may be taken as the melting point. This mean melting point is not equal experimentally to the melting point by Procedure A less 5°, because of the uneven heat exchange at higher temperatures which occasionally leads to overrunning the melting point as determined by Procedure A. If the melting point has been overrun considerably the solid will melt at temperature B, 10° are then subtracted from B, and the solid is again heated until $B - 10$ or C is attained. If the solid has not fused at C, the melting point is taken as the mean between B and C. However, the accuracy is such that only in rare instances is this third heating necessary.

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Determining Metallic Iron in Iron Oxides and Slags

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An investigation was undertaken to devise a new scheme which would eliminate the shortcomings of the mercuric chloride and copper sulfate methods of metallic iron analysis. Reliable results were obtained by using a feebly acetic acid solution of cupric potassium chloride under a carbon dioxide atmosphere.

The fundamental feature of the method is solution of iron by the cupric chloride of the reagent. The precipitated copper reacts with the excess cupric chloride to form cuprous chloride, which is removed by displacement with aluminum in hydrochloric acid solution, and the remaining ferrous chloride is titrated with potassium permanganate or with potassium dichromate if diphenylamine indicator is used.

The metallic iron was determined first with no other substances in the sample, then in the presence of iron oxides, mixed oxides, slags, etc. Comparison was made with the mercuric chloride method and with copper sulfate.

Experiments showed that cupric potassium chloride gives values that are comparable with mercuric chloride in the presence of magnetite, mixed oxides, and sponge iron, whereas copper sulfate gives high results. The new method does not give high results in the presence of ferrous sulfide or iron phosphide as both mercuric chloride and copper sulfate do, but, like mercuric chloride, gives high values with iron carbide. The method is limited to the determination of 0.1000 gram of metallic iron in the sample; if more is present, some of the ferrous chloride formed will oxidize during filtration and cause low results.

With basic open-hearth slags, cupric potassium chloride yields low values, but metallic iron recovery by this method is higher than with either copper sulfate or mercuric chloride.

THE determination of metallic iron in slags, ores, and mixed oxides of iron has been the object of investigations both in this country and abroad. As a result, two methods of analysis, the mercuric chloride (*10*) and the copper sulfate (*3*), are now in common use.

In the former, the iron is dissolved according to the reaction

$$Fe + 2HgCl_2 = FeCl_2 + 2HgCl$$

and the resulting ferrous chloride is titrated with potassium permanganate after the mercurous chloride has been removed by filtration. In the copper sulfate method, the iron is dissolved according to the reaction

$$Fe + CuSO_4 = FeSO_4 + Cu$$

and the resulting ferrous sulfate is titrated, as above, after the metallic copper and the excess of copper sulfate have been removed by displacement with aluminum in sulfuric acid solution.

Among the contributors to this subject are Williams and Anderson (*9*), who made tests on sponge-iron analysis and concluded that the mercuric chloride method is more accurate but that the copper sulfate method is more easily carried out. They state that both methods will indicate too large values of iron in the presence of ferrous sulfide or appreciable amounts of organic matter.

Ackermann (*1*) found that the mercuric chloride method gave satisfactory results in the presence of iron oxides, magnetite, and red ores, but that low values were obtained with brown ore (limonite), bog iron, and oölitic brown ores. In addition, he found that mixtures of reduced iron with ferrous sulfide, ferric phosphide, or iron carbide gave high recoveries for metallic iron. Oakley and Krantz (*5*) noted the high value caused by the presence of iron sulfides when making analyses for metallic iron by the copper sulfate method. More recently, Petzold (*6*) has made a detailed investigation of the mercuric chloride method and states that this method alone gives satisfactory results in determining metallic iron in the presence of its oxides, but that it yields low values in the presence of slags.

The New Method

The shortcomings of the methods discussed above suggested that the use of another reagent might lead to more satisfactory results. Examination of other possibilities suggested the trial of cupric potassium chloride. The employment of this reagent in chemical analysis is not new and its use was suggested as far back as 1871 by Meineke (*4*) in the gravimetric determination of sulfur. However, the hydrochloric acid solution in which this reagent was used attacks iron oxides and ferrous sulfide. After making a number of trials of cupric potassium chloride solution containing various acids, satisfactory results were obtained by using a faintly acetic acid solution of the reagent under a carbon dioxide atmosphere.

The fundamental feature of the method is solution of the iron by the cupric chloride of the reagent, according to the reaction

$$Fe + CuCl_2 = FeCl_2 + Cu$$

During the action, in which the potassium chloride probably aids in the solution of the copper by the formation of a double salt, the precipitated copper reacts with the excess cupric chloride to form cuprous chloride

$$Cu + CuCl_2 = 2CuCl$$

which is then removed by displacement with aluminum in hydrochloric acid solution and the remaining ferrous chloride is titrated with potassium permanganate, or alternatively, with potassium dichromate if diphenylamine indicator is used. As the result of a series of experiments the procedure shown below was decided upon as the most suitable.

PREPARATION OF REAGENT. The solution to be used can be prepared by dissolving 100 grams of cupric potassium chloride dihydrate in 1 liter of distilled water, or by dissolving 53.35 grams of C. P. cupric chloride dihydrate and 46.65 grams of C. P. potassium chloride in 1 liter of distilled water. The latter method is recommended if trouble is encountered in obtaining the former reagent with a sufficiently low iron content. However, the author's experience indicates that the iron content of the other salts, particularly the cupric chloride dihydrate, also varies considerably, and the reagents should be selected carefully to avoid excessively large blanks. The exact formula weight relationships used to make up the cupric potassium chloride solution are not necessary to obtain valid results—for example, reliable values were obtained when the solution was made up on the

gram molecular weight basis of 1.27 $KCl.CuCl_2.2H_2O$ instead of $2KCl.CuCl_2.2H_2O$.

Outline of Method. Satisfactory solution of the metallic iron is obtained if the sample is powdered to pass through a 100-mesh sieve.

A 1- to 2-gram sample (which should not contain more than 0.1000 gram of metallic iron) is weighed out and transferred to a 125-ml. Erlenmeyer flask that has been swept free of air by carbon dioxide. Thirty milliliters of the cupric potassium chloride are poured into a glass graduate, 5 drops of glacial acetic acid are added from a pipet, and a stream of carbon dioxide is bubbled through the solution for a few minutes. The reagent is then decanted into the Erlenmeyer flask and agitated at room temperature for 30 minutes with a steady stream of carbon dioxide bubbling through the solution. The flask should be swirled occasionally to prevent caking of the sample, since this retards solution of the iron.

The contents of the flask are then filtered at once through a No. 40 Whatman filter paper, or its equivalent, into a 300-ml. Erlenmeyer flask under light suction, using a platinum cone, and the whole is washed thoroughly with cool distilled water, keeping the volume from 125 to 150 ml. About 1 gram of aluminum shavings (iron-free or low in iron) and 6 ml. of 1 to 1 hydrochloric acid are added, and the solution is boiled gently under an atmosphere of carbon dioxide until it is colorless and all the copper is displaced. While still under a carbon dioxide atmosphere the flask with its contents is cooled in ice water. The colorless solution is filtered through a No. 42 Whatman filter paper, or its equivalent, into a 500-ml. Erlenmeyer flask under light suction, using a platinum cone, and the residue is washed thoroughly with cold distilled water. Next, the solution is titrated with standard potassium permanganate to a faint pink end point after 10 ml. of Zimmermann-Reinhardt reagent are added. The blank is determined by proceeding through all the steps of the method, using the same amounts of reagent and solution, but bubbling carbon dioxide through the solution for only a few minutes instead of for 0.5 hour.

Table I. Recovery of Metallic Iron in Absence of Foreign Material

Electrolytic Iron Present	$HgCl_2$ Method		$KCuCl_3$ Method		$CuSO_4$ Method	
Gram	*Gram*	%	*Gram*	%	*Gram*	%
0.1000	0.1002	100.2	0.0999	99.9	0.1002	100.2
0.1000	0.0996	99.6	0.0997	99.7	0.0999	99.9
0.1500	....	...	0.1488	99.2	....	...
0.2000	....	...	0.1958	97.9	....	...

Experimental Results

To determine the reliability of the method, it was decided to start with electrolytic iron and from it proceed to iron oxide, mixed oxides, slags, and so on. Comparison was usually made with the mercuric chloride method because it is considered the standard, but also, in some instances, with the copper sulfate method to compare the behavior of all three.

Table I illustrates the recovery of electrolytic iron by the three methods. As shown in columns 4 and 5, the recovery of electrolytic iron is decreased in the cupric potassium chloride method if the weight of sample exceeds 0.1000 gram.

With this information as a starting point, it was decided to determine the recovery of metallic iron in the presence of magnetite. Bureau of Standards magnetite ore 29 was selected because previous analyses by both the mercuric chloride and cupric potassium chloride methods had shown it to be free from metallic iron. To obtain a representative sample, 0.4380 gram of electrolytic iron was mixed well with 19.5620 grams of the ore, and 1-gram samples of this mixture were analyzed by the cupric potassium chloride method as shown in Table II.

To test the reagent on mixed oxides of iron, such a sample was prepared by melting c. p. ferric oxide in an Armco iron crucible and pouring the product into a copper mold to cool it quickly. The resulting ingot was analyzed by the sulfuric-hydrofluoric acid method of Soule (*8*) and found to contain 87.5 per cent of ferrous oxide. Additional samples containing less ferrous oxide were made by melting the 87.5 per cent material with varying

Table II. Metallic Iron Recovery in Presence of Magnetite Ore

Cupric Potassium Chloride Method

Mixed Sample	Electrolytic Iron Taken	Electrolytic Iron Recovered	
Gram	*Gram*	*Gram*	%
1.0000	0.0219	0.0218	99.5
1.0000	0.0219	0.0215	98.2
1.0000	0.0219	0.0221	100.8

amounts of c. p. ferric oxide in a thick-walled platinum crucible under a nitrogen atmosphere containing 0.3 per cent of oxygen. These products were then analyzed for metallic iron by the three different methods. Analyses by these three reagents were also made on sponge iron and the 87.5 per cent iron oxide to which electrolytic iron had been added.

The results are compiled in Table III. As indicated in column 6, the mixed oxides and the sponge iron gave consistently high results with copper sulfate, whereas good agreement was obtained with mercuric chloride and cupric potassium chloride on the same material.

Table III. Metallic Iron Recovery in the Presence of Mixed Oxides

Sample	Sample Weight	Metallic Iron Added	Method Used	Metallic Iron Found	
%	*Grams*	*Gram*		*Gram*	%
Iron oxide, FeO					
87.5	2.0000	....	$HgCl_2$	0.0204	1.02
	2.0000	....	$HgCl_2$	0.0204	1.02
	1.0000	....	$KCuCl_3$	0.0102	1.02
	1.0000	....	$KCuCl_3$	0.0102	1.02
	1.0000	....	$CuSO_4$	0.0151	1.51
	1.0000	....	$CuSO_4$	0.0148	1.48
82.8	2.0000	....	$HgCl_2$	0.0818	4.09
	2.0000	....	$HgCl_2$	0.0824	4.12
	1.0000	....	$KCuCl_3$	0.0407	4.07
	1.0000	....	$KCuCl_3$	0.0409	4.09
	2.0000	....	$CuSO_4$	0.1147	5.73
	2.0000	....	$CuSO_4$	0.1158	5.79
77.35	1.0000	....	$KCuCl_3$	0.0011	0.11
	1.0000	....	$KCuCl_3$	0.0011	0.11
63.95	1.0000	....	$HgCl_2$	0.0008	0.08
	1.0000	....	$KCuCl_3$	0.0005	0.05
27.3	1.0000	....	$HgCl_2$	0.0016	0.16
	1.0000	....	$KCuCl_3$	0.0016	0.16
87.5	1.0000	0.1000	$HgCl_2$	0.1100	99.85 } [a]
	1.0000	0.1000	$KCuCl_3$	0.1098	99.6 } [a]
	1.0000	0.1000	$CuSO_4$	0.1153	100.1 } [a]
Sponge iron[b]	0.2000	....	$HgCl_2$	0.1582	79.10
	0.2000	....	$HgCl_2$	0.1582	79.10
	0.2000	....	$KCuCl_3$	0.1563	78.15
	0.1000	....	$KCuCl_3$	0.0792	79.20
	0.2000	....	$CuSO_4$	0.1725	86.20
	0.2000	....	$CuSO_4$	0.1725	86.20

[a] Total weight of metallic iron which could be recovered in each case is the sum of the 0.1000 gram in column 3 plus grams of iron per 1.000 gram of sample found for 87.5 per cent ferrous oxide material by methods of analysis shown at top of column 5.

[b] Furnished by Pacific Experiment Station of Bureau of Mines.

To supplement the study of the new reagent as a means of analysis for metallic iron among reduced products, the possible interfering effects of ferrous sulfide, iron-phosphorus alloys, and iron carbide were investigated. The ferrous sulfide prepared and furnished by the North Central Experiment Station was 99.5 per cent pure by chemical analysis. The iron-phosphorus alloys were prepared by adding red phosphorus to molten electrolytic iron in a nitrogen atmosphere. Iron carbide was prepared according to the procedure of Brodie, Jennings, and Hayes (*2*). It was approximately the composition required for the compound Fe_3C, a slight excess (about 0.04 per cent) of carbon being present. Enough iron carbide was not obtained to check its reaction with mercuric chloride and copper sulfate.

Table IV contains the results of this part of the investigation. From column 4 it will be seen that the samples which contain an excess of phosphorus beyond that necessary to form Fe_3P, a compound containing 15.62 per cent of phosphorus, show very little attack by cupric potassium chloride.

TABLE IV. REACTIONS OF DIFFERENT METHODS WITH POSSIBLE INTERFERING SUBSTANCES

Sample	Sample Weight	Reaction as Metallic Iron $HgCl_2$ method	$KCuCl_3$ method	$CuSO_4$ method
	Gram	%	%	%
Ferrous sulfide	1.0000	3.76	0.05	0.83
Iron-phosphorus alloys				
5.52 % P	1.0000	..	9.40	..
6.18 % P	1.0000	..	10.05	..
16.25 % P	1.0000	1.51	0.03	..
20.6 % P	1.0000	..	0.05	..
22.16 % P	1.0000	..	0.05	..
Iron carbide	0.1000	..	11.32	..

Conversely, the samples which contain less than the required amount of phosphorus show the presence of considerable amounts of metallic iron. The attack upon ferrous sulfide and iron phosphide by mercuric chloride is in agreement with the results of Ackermann.

The trial of the three methods with basic open-hearth slags comprises the final phase of the investigation. Two series of slags, taken progressively at various stages of the heat, were obtained from the Carnegie-Illinois Steel Corporation and the Wheeling Steel Corporation. Each complete sample was ground in a planetary grinder until it was fine enough to pass through a 100-mesh screen sieve. It was then thoroughly mixed on glazed paper to ensure homogeneity and analyzed in the usual manner for metallic iron and ferrous oxide.

As can be seen in Table V, columns 3, 4, and 5, no reliable values of metallic iron could be obtained. For this reason, the percentage of ferrous oxide as determined by analysis was not corrected for the metallic iron value—that is, the figures for ferrous oxide given in column 7 include the iron calculated to FeO.

Furthermore, it was not possible to duplicate results by consecutive analyses on the same slags. Confirmation of the presence of metallic iron in all the slags was obtained by treating each with a neutral solution of copper sulfate and also with cupric potassium chloride solution at room temperature with the resultant plating out of metallic copper. As further evidence, each sample of slag was checked thermomagnetically, with apparatus that has previously been described (*7*), and found to contain considerable amounts of free iron. Column 6 gives the results of the magnetic tests. The values shown were obtained by subtracting the relative susceptibility at 800° C. from the value determined at 600° C. As at 600° C. metallic iron alone among the constituents of the slag remains in the ferromagnetic range, it is responsible for the relatively high susceptibility of the sample. At 800° C. iron also has lost its ferromagnetism, so that the average susceptibility of the sample is greatly decreased. Hence, the difference between susceptibility at 600° C. and at 800° C. is indicative of the amount of metallic iron present. The figures in column 6 are differences, not of actual susceptibility, but of unconverted readings that are roughly proportional to susceptibility. The general correlation between the figures of columns 6 and 4 is evident.

These variable results made it necessary to approach the problem from a different angle.

A portion of slag III was passed several times through a direct current magnetic separator in which the field strength was approximately 250 oersteds and the lifting power was such as to separate particles with an effective permeability of about 3 or more from those of lower permeability. (The formula for the force on a particle in a magnetic field is $f = mxH\ dH/dy$ when f is the force in dynes, m is the mass of the particle in grams, x is the mass susceptibility in c. g. s. units, and H and dH/dy are the field strength and field gradient, respectively, in which the particle is located.) This operation separated the sample into two parts, of which the more magnetic one contained most of the metallic iron. Both the nonmagnetic and magnetic portions were analyzed in the usual manner for metallic iron.

A small amount of metallic iron was found in the nonmagnetic portion upon analysis with copper sulfate and cupric potassium chloride, whereas its presence could not be detected with mercuric chloride. Electrolytic iron in the form of powder or small particles was then added to the nonmagnetic portion and analysis made to check the recovery. The results are compiled in Table VI.

Column 6 shows that the recovery of metallic iron by all three methods is low in the presence of basic open-hearth slags. With cupric potassium chloride the recovery approximates 60 per cent and with copper sulfate, about 25 per cent, while mercuric chloride gives by far the lowest percentage of all. In addition to the data that are tabulated, similar results were obtained in a number of analyses carried out to check the technique.

TABLE V. METALLIC IRON VALUES OBTAINED IN BASIC OPEN-HEARTH SLAGS

Slag	Slag Weight	Present as Metallic Iron $HgCl_2$ method	$KCuCl_3$ method	$CuSO_4$ method	Relative Susceptibility Due to Iron	FeO
	Grams	%	%	%	*Arbitrary units*	%
Carnegie-Illinois						
I	2.0000	None	0.28	0.24	68	13.55
II	2.0000	None	0.85	0.65	142	12.53
III	2.0000	0.11	2.62	2.63	272	23.30
IV	2.0000	None	2.43	1.90	257	20.52
V	2.0000	None	1.87	1.56	240	18.50
VI	2.0000	0.05	2.19	1.50	250	19.24
Wheeling						
A	2.0000	0.23	0.82	1.51	97	11.49
B	2.0000	None	0.90	0.81	149	13.15
C	2.0000	None	0.90	0.96	113	13.25
D	2.0000	0.11	0.63	1.12	74	10.82
E	2.0000	None	0.40	1.18	60	10.11
F	2.0000	None	0.41	0.55	64	10.54
G	2.0000	None	0.76	0.98	82	14.18
H	2.0000	None	0.79	1.42	85	13.85
I	2.0000	None	0.72	0.70	76	12.05

TABLE VI. METALLIC IRON VALUES FOUND IN NONMAGNETIC PORTION OF A BASIC OPEN-HEARTH SLAG UPON ADDITION OF ELECTROLYTIC IRON

Slag	Slag Weight	Metallic Iron Added	Method Used	Metallic Iron Found	
	Grams	*Gram*		*Gram*	%
III (nonmagnetic portion)	2.0000	.....	$HgCl_2$	None	None
	2.0000	.....	$KCuCl_3$	0.0022	0.11
	2.0000	.....	$CuSO_4$	0.0030	0.15
III (magnetic portion)	0.5000	.....	$HgCl_2$	0.2173	43.45
	0.2500	.....	$KCuCl_3$	0.1246	49.84
III (nonmagnetic portion)	2.0000	0.1000 (powder)	$HgCl_2$	0.0005	0.05
	2.0000	0.0500 (powder)	$HgCl_2$	0.0005	0.05
	2.0000	0.1000 (particles)	$HgCl_2$	0.0005	0.05
	2.0000	0.0500 (particles)	$HgCl_2$	0.0005	0.05
	2.0000	0.1000 (powder)	$KCuCl_3$	0.0591	59.1
	2.0000	0.0500 (powder)	$KCuCl_3$	0.0311	62.2
	2.0000	0.1000 (particles)	$KCuCl_3$	0.0539	53.9
	2.0000	0.0500 (particles)	$KCuCl_3$	0.0299	59.8
	2.0000	0.1000 (powder)	$CuSO_4$	0.0271	27.1
	2.0000	0.1000 (powder)	$CuSO_4$	0.0246	24.6

Summary

The cupric potassium chloride method has been examined as a means for analysis of metallic iron. Experiments show that it gives values that are comparable with mercuric chloride in the presence of magnetite, mixed oxides, and sponge iron, whereas copper sulfate under these conditions gives high results. It has the advantage that it does not give high results in the presence of ferrous sulfide or iron phosphide as both mercuric chloride and copper sulfate do but it has the disadvantage, like mercuric chloride, of giving high values with iron carbide.

The method is limited to the determination of 0.1000 gram of metallic iron in the sample, because if more is present, some

of the ferrous chloride formed in the reaction will oxidize during filtration and cause low results. With basic open-hearth slags, cupric potassium chloride yields low values, but recovery of metallic iron by this method is higher than with either copper sulfate or mercuric chloride.

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Methods for Clarifying Oxidized or Used Mineral Oils and Determining Sludge

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AFTER an oil has from use or from artificial oxidation become contaminated with solids or so-called "undissolved sludge" and with "dissolved sludge", it is frequently desirable to obtain a portion of this deteriorated oil in a clarified condition, free of undissolved sludge. It is often informative regarding changes which have occurred in the oil to determine dissolved sludge in the clarified sample or to compare viscosity, neutralization number, saponification number, resin content, ash, and carbon residue of the original oil, the oil containing the total sludge, and the clarified oil.

Clarifying Oxidized Mineral Oils

Fenske and his co-workers (*2, 3*) have made valuable contributions to our knowledge of the composition and methods of analysis of the oxidation products of mineral oils. They mention the use of Jena sintered-glass filters of fine porosity (G-4) for clarifying oxidized oil. Levin and Towne (*6*) suggest the employment of surgical absorbent cotton to filter out undissolved sludge. Davis and co-workers (*1*) point out that because oil may contain insolubles of every degree of fineness from coarse particles to colloid suspensions of such size that the particles will pass through cotton and an asbestos mat in a Gooch crucible, there seems at present to be no satisfactory definition of "oil-insolubles". Such terms as "oil-insolubles", "undissolved sludge", "dissolved sludge", and "resins" are in fact defined by the methods utilized for their separation, but no procedures now in use are able to effect 100 per cent separation of these materials if a literal interpretation of this terminology is applied.

Apparatus. Clarification is effected in the author's laboratory by hot-pressure filtration through Seitz asbestos filtering disks of the S-3 grade. The assembled apparatus is shown in Figure 1. Two 140-mm. diameter filter disks are used simultaneously with a disk of filter paper between the lower asbestos disk and the two perforated steel support plates, supported by the corrugated surface of the filter base. (The filter base and perforated plates may be obtained as a unit of the No. 14 laboratory filter of American Seitz Filter Co., 480 Lexington Ave., New York, N. Y., where Seitz S-3 filter disk may also be secured.) Drawing the filter base up into the recessed section of the flange ring by means of the wing-head threaded bolts and the horizontal support lugs seals the disks at the edges. The pressure cylinder was made by welding a piece of heavy steel tubing to the flange ring. A head with three threaded connections in it was welded on the top of the steel tubing to close the upper end. The three connections are used for a pressure gage, inlet gas pressure, and a threaded plug.

Procedure: The desired charge of oil is placed in the assembled apparatus through the plug connection. The plug is replaced, the inlet gas pressure opening connected to a suitable source of pressure such as air or nitrogen, and the assembled apparatus placed in an oven which will bring it to the desired clarification temperature. When the desired temperature is reached, the gas pressure is applied—usually 1.05 kg. per sq. cm. (15 pounds per sq. inch). The efflux clarified oil is collected in a receiver placed under the filter drain tube. If desired, the receiver may be put outside the oven by providing a hole in the bottom for the drain tube.

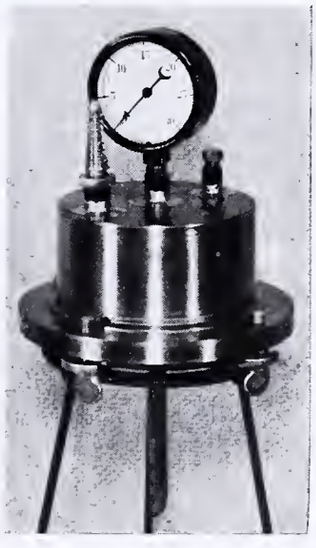

Figure 1

Discussion. As pressure filtration is used, the method provides a means of obtaining a clarified sample unchanged except for being freed of undissolved sludge at the filtration temperature.

With most oils of the S. A. E. No. 20 or 30 grade, 10 or 15 minutes of filtration time are required to obtain 200 ml. of clarified oil where the operation is carried out at 93.3° C. (200° F.) and at 33 kg. (15 pounds) pressure.

The clarity of the filtered oil is influenced by the amounts of soluble, dark-colored oxidation products. In cases of highly stable oils clarified by this method, ten-point print can easily be read through a (51-mm.) 2-inch layer of the oil.

Determination of Sludge

Among the types of filters suggested for laboratory determination of sludge are Alundum crucibles, filter paper, porous porcelain, and sintered glass. Of these media sintered glass seems to be the most satisfactory. The most

commonly used type of laboratory filter for sludge determination at present is believed to be the Gooch crucible with an asbestos mat prepared as needed from asbestos fiber.

Using asbestos mats in Gooch crucibles, a wide variety of recommendations have been made regarding preparation of the mats, dilution ratio, oil solvents, and conditions and time of standing before filtration (*3*, *6*, *7*, *8*). Difficulty is often experienced in securing a mat retentive enough to give a clear filtrate.

Levin and Towne (*6*) classify sludge in used oils as undissolved and dissolved. They have described a method for determining undissolved sludge based on the difference between percentage of commercial pentane-insoluble material in the original used oil and in a sample of a separate portion of the original oil after clarification. They have indicated that dissolved sludge may be precipitated, filtered out, and weighed as sludge where oil-dilution solvents are used, especially at high-dilution ratios. Hall, Levin, and McMillan (*5*) recommend for dissolved sludge determination a procedure based on the propane-insoluble material in a clarified sample. Ludeman (*7*) has recently reported on an investigation of a number of factors influencing the data obtained in sludge determinations. He outlines a standard procedure, dividing the individual sludge components into seven classes of materials mainly on the basis of solubility in various suitable solvents. Gruse and Livingstone (*4*) have summarized the source and chemical and physical nature of sludge deposits. As pointed out by Davis, Lincoln, Byrkit, and Jones (*1*), in the determination of petroleum naphtha–insoluble material, widely different results may be obtained, depending upon the fineness of the filter medium employed. Other probable factors are the thickness of the sludge layer formed in the crucible and the particle size distribution of the sludge.

FIGURE 2

APPARATUS. The apparatus developed by the author, which consists essentially of a brass filter funnel body of special design and an aluminum cylinder, is shown assembled in Figure 2. Seitz S-3 grade asbestos disks 80 mm. in diameter are used as the filter medium. A removable, perforated steel plate situated in a recessed portion of the base ring supports the filter disk. Four evenly spaced, top-threaded studs are set into the base ring, so that the wing nuts that are used to tighten down the upper recessed ring, pulling it against the aluminum cylinder, cause the cylinder to seat on the Seitz disk and seal the lower edge of the liquid container. The bottomless aluminum cup is made of 17-gage (1.125-mm., 0.045-inch) tubing. The lower portion of the cup is 75 mm. in diameter and 12 mm. high. A brass band approximately 18 mm. wide is soldered around the outside of the base ring, about which the filter assembly is constructed. The purpose of the reservoir formed between this band and the aluminum cup is to hold fresh liquid for outside to inside washing of the edge of the filter disk.

METHOD. Any suitable oil solvent, such as A. S. T. M. precipitation naphtha or commercial pentane, may be used for diluting the sample of oil containing the sludge. The author ordinarily dilutes 100 ml. of the oil with 200 ml. of precipitation naphtha. The results are expressed in grams of sludge per 100 ml. of oil sample. A quantitative filter paper disk, the Seitz disk, and the aluminum cylinder are placed in a hot-air oven at 105° C. for drying to constant weight. After cooling and weighing, these three pieces are assembled in the apparatus with the filter paper between the Seitz disk and the support plate. The cylinder is centered on the Seitz disk with its large end resting on the disk. The upper ring is placed over the cylinder and drawn down by means of the wing nuts. The whole assembly is then put on a suction flask, as shown in Figure 2, and the filtration is conducted in the conventional manner. After filtration is complete, the sludge which is deposited on the disk is well washed with the dilution solvent. After thorough washing, to remove adhering oil from the sludge and the inside of the cylinder, some of the solvent is placed in the outer reservoir and drawn through by suction, washing the edge of the disk from outside in and down. The filter paper, the Seitz disk, and the filtering cylinder are then removed as a unit by placing a wide-bladed spatula under the filter paper. This unit is dried, cooled in a desiccator, and reweighed. The increase in weight represents the amount of sludge.

This same apparatus may be used to determine the portion of the sludge which is insoluble in a particular solvent, such as precipitation naphtha or pentane, and soluble in another solvent, such as chloroform or carbon disulfide.

TABLE I. SLUDGE OBTAINED FROM USED OIL

Used Oil	Seitz S-3	G-4 Sintered Glass	Gooch Asbestos Mat
A	1.10	1.01	1.04
	1.05	1.03	1.06
B	0.84	0.82	0.81
	0.83	0.81	0.84
C	0.56	0.55	0.54
	0.55	0.54	0.54
D	0.34	0.33	0.32
	0.30	0.32	0.31
E	0.54	0.55	0.50
	0.53	0.56	0.50

Table I shows a comparison of the precipitation naphtha–insoluble sludge obtained in five different samples of used oil by the Seitz disk filtration method, Jena sintered-glass crucibles, and asbestos fiber mats prepared in Gooch crucibles. A dilution ratio of 2 to 1 was used in all these tests. The determinations on each oil by each method were made in duplicate; the results are expressed in percentage of sludge by weight.

ADDITIONAL APPLICATIONS. Sludge solubilities at different oil temperatures may be studied by placing the filter assembly containing a quantity of undiluted sludge-oil mixture in a suitable oven and carrying on the filtration at the desired temperature. To obtain a clear filtrate under these conditions, it has been found advisable to use simultaneously two Seitz disks in the apparatus.

Another indicated application is the use of the apparatus to measure the solubility of sludge in so-called "sludge solvents".

DISCUSSION. Where a large enough sample (usually 25 ml. is the minimum needed) of the used oil is available for this method, it seems to possess the following advantages over conventional procedures: saving of time owing to the elimination of some weighings, rapid filtration through the large filtering surface, use of a dilution ratio so low that dissolved sludge is not likely to precipitate, and, if desired, saving of the sludge deposit with the disk for reference or for comparison. No difficulty in securing clear filtrates has ever been experienced and it has not been necessary to allow the customary standing time of the diluted sample of 3 hours or more. In the Seitz disk method the diluted sample may be filtered immediately after dilution.

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Thiocyanate Method for Iron

A Spectrophotometric Study

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INASMUCH as the thiocyanate method for iron is generally accepted as official by the technical organizations having standards of analytical practice (*1–3*, *22*) and the procedure seems to be inferior to certain other colorimetric methods for this element, the operating conditions now recommended were critically studied. In addition to investigating various proposed modifications of the original procedure, an attempt has been made to evaluate the method in comparison with several other procedures recently suggested for the same determination.

Since the thiocyanate method is very old, having been proposed in 1837 by Ossian (*14*), its literature is voluminous. Stokes and Cain (*18*) pointed out many of the errors and suggested as the reagent thiocyanic acid stabilized with mercuric thiocyanate, adding potassium persulfate to oxidize the iron. Hydrogen peroxide and potassium permanganate have been used for the same purpose (*15*). Bernhard and Drekter (*5*) suggested extracting the colored compound by means of a mixture of the monobutyl ether of ethylene glycol and ethyl ether. The extract has a more intense color and does not fade for 24 hours. Addition of acetone increases the sensitivity of the reagent and decreases the error due to phosphates (*10*). Winsor (*24*) used 2-methoxyethanol for the same purpose.

Two mixtures of salts have been proposed as solutions for permanent color standards. Jackson (*9*) used cobaltous chloride and potassium chloroplatinate, while Velichkovskaya (*23*) used cobaltous nitrate and ferric chloride.

At present the nature of the reaction between ferric and thiocyanate ions remains controversial. The work of Stokes and Cain (*18*), and later that of Schlesinger and Van Valkenburgh (*16*), seem to indicate the presence of an anion, $Fe(SCN)_6^{---}$. Opposed to this concept is the work of Møller (*13*) and of Bent and French (*4*) who believe the color is due to a cation, such as $Fe(SCN)^{++}$ or $Fe(SCN)^{+}$.

Apparatus and Methods

All color measurements were made with a General Electric photoelectric spectrophotometer, the spectral band width being 10 mμ and the cell thickness 1.000 cm. Typical spectral transmission curves were selected for calculating tristimulus values by means of Hardy's ten selected ordinates (*7*) and Swank's calculator (*21*). Calculation of the extent of the interference by diverse ions was facilitated by a color slide rule (Keuffel and Esser).

A stock solution containing 100 p. p. m. of iron was made by dissolving pure wire in 10 ml. of 6 *N* nitric acid and diluting to 2 liters. The directions of Snell (*17*) and Yoe (*25*) were followed for the solution of thiocyanic acid. Material from the General Chemical Company was used for a solution of ammonium thiocyanate, as the very small amount of iron in this product could be extracted easily from a slightly acidic solution with four or five portions of a 5 to 2 mixture of amyl alcohol and ethyl ether. The resulting solution, if slightly ammoniacal, is stable in the dark for several weeks.

Solutions of recrystallized hexaquocobaltous chloride and potassium chloroplatinate were prepared, according to the directions of the American Public Health Association (*1*) for use as color standards. Other specially prepared materials used for this purpose, together with the concentration of the solutions, are listed in Table I.

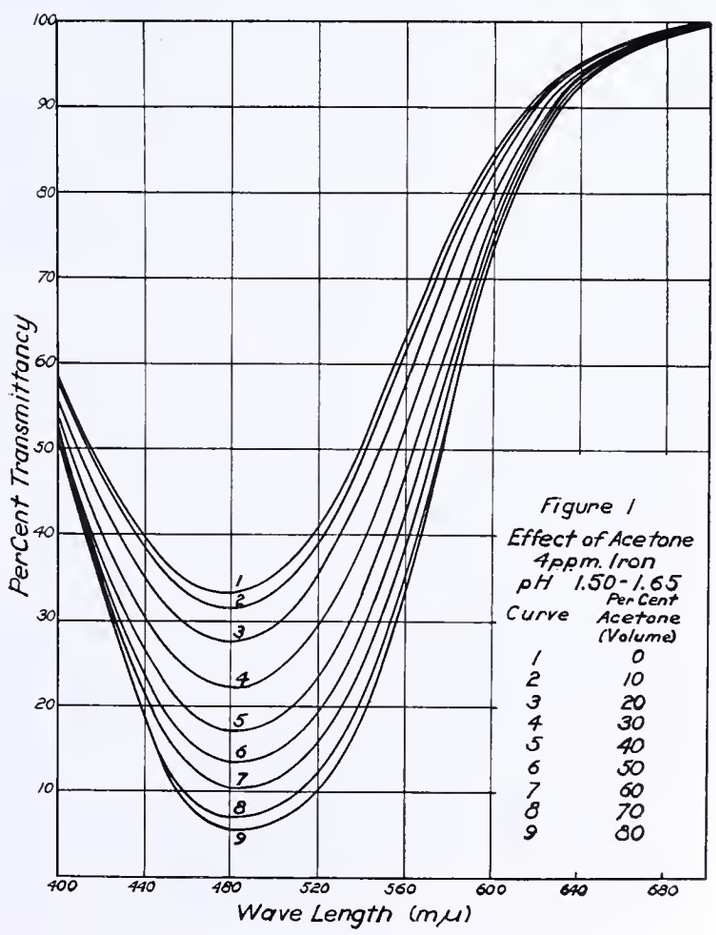
During the work on the variable factors studied several variations were used in preparing the solutions to be measured. After deciding upon the best conditions, an operating routine was established. Five milliliters of a solution containing 0.020 mg. of iron per ml. were measured into a 50-ml. volumetric flask. To this were added, in order, 5 ml. of 6 *N* nitric acid, 2 ml. of 20 per cent ammonium thiocyanate solution, and 30 ml. of acetone. The flask was filled to the mark, the solution mixed, and the spectrophotometric curve determined within 15 minutes. Diverse ions, when used, were added prior to the thiocyanate. Measurement of pH values of the solutions was made with a glass electrode assembly (*12*).

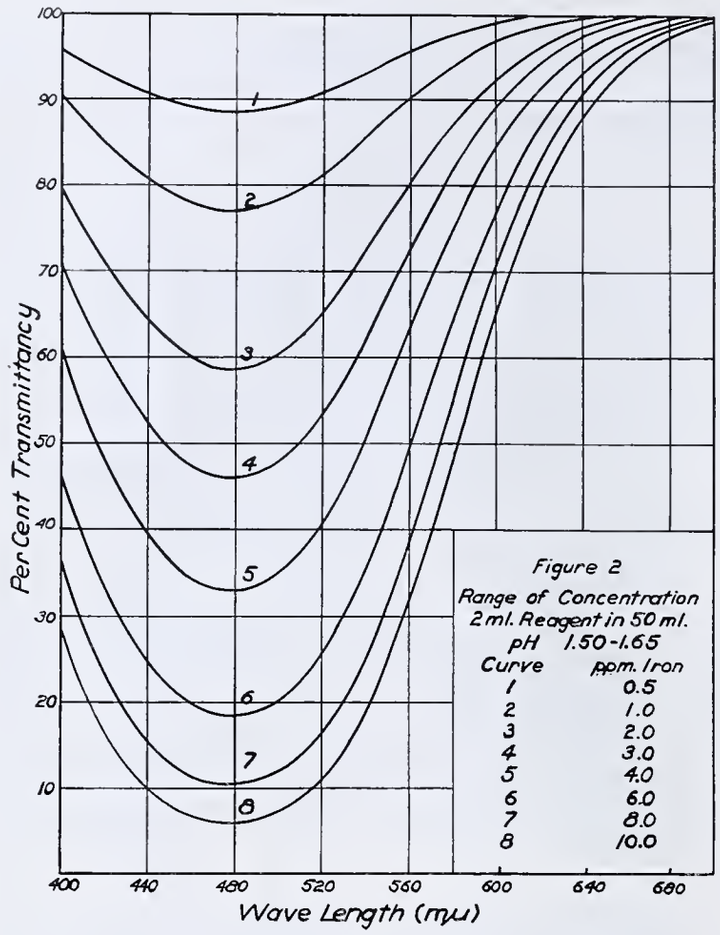
TABLE I. COMPOSITION OF PERMANENT STANDARDS

Fe Concn.	Materials in Solution (Mixture Diluted to 100 Ml.) 0.201 *M* $CoCl_2$	0.00823 *M* K_2PtCl_6
P. p. m.	*Ml.*	*Ml.*
0.2	1.20	2.00
0.5	3.05	5.55
1.0	7.30	11.50
2.0	20.00	22.60
	0.250 *M* $CoCl_2$	0.0167 *M* $FeCl_3$
0.5	4.00	16.00
1.0	7.00	22.00
2.0	14.00	8.00
	0.010 *M* $Co(NH_3)_6(NO_3)_3$	0.010 *M* $Co(NH_3)_5(H_2O)Cl_3$
0.2	4.00	0.40
0.5	8.50	1.25
1.0	16.00	4.50
2.0	31.00	12.00

Color Reaction

SELECTION OF REAGENT. The depth of color developed depends upon the amount of reagent used, whether it is thiocyanic acid or ammonium thiocyanate. The ammonium salt was used, because it gives a much deeper color than the acid





stabilized with mercuric thiocyanate, and is easily freed of any iron. The higher sensitivity is presumably due to the fact that thiocyanic acid and mercuric thiocyanate form a complex, $H_2Hg(SCN)_4$. Increasing amounts of reagent shifted the peak of the absorption band toward the red. For 0.5 ml. the minimum is at 460 mμ and for 5.0 ml. it is at 480 mμ.

Effect of Solvent. Addition of acetone as solvent increases the sensitivity of the method, the magnitude of the effect being shown in Figure 1. This change has been attributed to the lower dielectric constant of the acetone solution. Also, the error due to fading decreases from about 50 per cent in 20 hours for a water solution to about 10 per cent in the same time for one containing 60 per cent of acetone by volume. Here, as elsewhere in this article, per cent error refers to the calculated concentration of iron.

Effect of pH and Kind of Acid. As trials with sulfuric acid seemed to reveal no advantages, most of the work was confined to nitric and hydrochloric acids. The latter is specified in the A. P. H. A. method, but nitric acid seems preferable.

With hydrochloric acid and the thiocyanic acid reagent, the optimum pH range is 1.3 to 1.8, the color being less intense in either higher or lower acidities. With ammonium thiocyanate and hydrochloric acid the best range is 1.4 to 2.4 in water alone, or 1.2 to 1.7 in 60 per cent acetone solution.

With nitric acid and ammonium thiocyanate the acidity should be maintained below pH 1 both in water and in acetone solutions. The lower limit was not accurately found; but since strong nitric acid itself gives a color with thiocyanate, the acidity should not be much greater than 1 *N*.

Range of Concentration and Conformity to Beer's Law. The curves in Figure 2 show that the workable range of concentration in water, with hydrochloric acid, is 0.1 to 10.0 p. p. m. in a 1-cm. cell. A plot of the logarithm of the transmittancies at 478 mμ against concentration shows that Beer's law holds, at least up to 10.0 p. p. m. With nitric acid and acetone the range is 0.05 to 5.0 p. p. m., and the law holds if the pH is maintained in the range 1.2 to 1.5, but not for higher acidities.

Effect of Oxidizing Agents. The effect of persulfate, periodate, and hydrogen peroxide was studied in the presence of hydrochloric acid and acetone. The first two cannot be used, as they immediately oxidize the reagent and produce a yellow hue. Excess hydrogen peroxide produces the same effect on standing; if carefully controlled, this oxidant may be used. Nitric acid is the most practical, since it serves both as the oxidizing agent and for controlling the acidity.

Extraction of Colored Compound. The color of the solution obtained by extracting the ferric thiocyanate complex with a mixture of equal volumes of amyl alcohol and ethyl ether has about the same intensity as that for a nitric acid–acetone solution of the same concentration of iron. The hue of the extract is more purplish, the peak of the absorption band being at 500 mμ. Since extraction did not seem to improve the method appreciably, this complication was not continued.

Effect of Diverse Ions. A study was made of the effect of many of the more common ions upon the development of the iron-thiocyanate color. Unless otherwise stated, the measurements are for a solution containing 60 per cent acetone.

Cations. Of the 27 cations studied only the aluminum, ammonium, beryllium, cerous, lithium, magnesium, manganous, potassium, and sodium ions do not interfere more than 2 per cent when present in concentrations 250 times that of the iron.

Barium, calcium, lead, strontium, and zirconyl ions interfere because of the small solubility of their nitrates in the acetone solution.

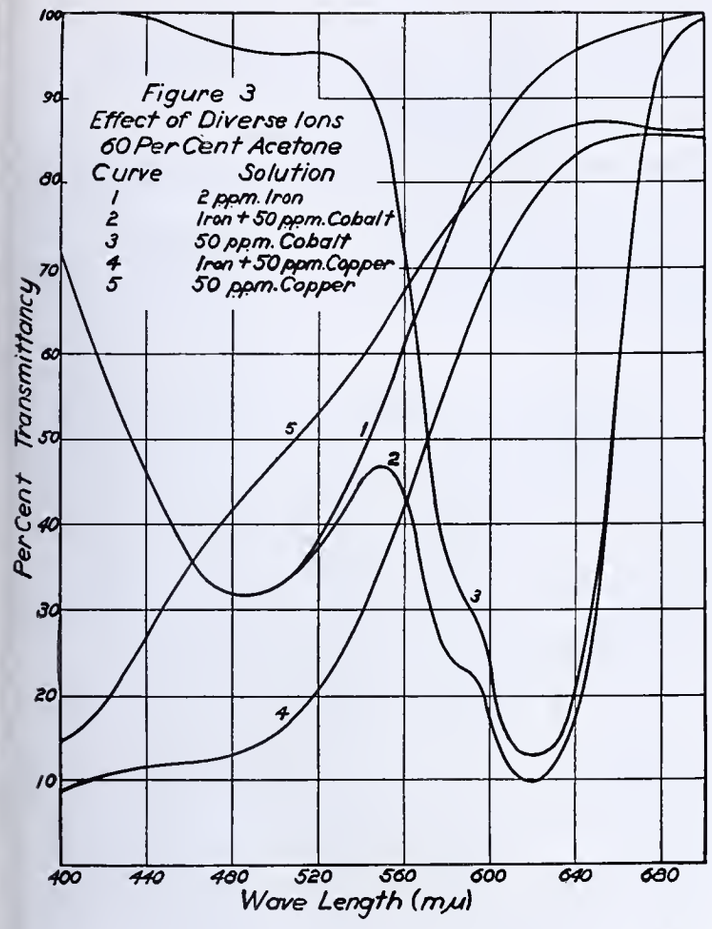
Silver and mercurous ions form insoluble thiocyanates and must, therefore, be entirely absent. In contrast, several cations interfere by forming soluble colorless complexes with thiocyanate. The presence of these ions, which include antimonous, cadmium, mercuric, and zinc, leads to a distinct bleaching of the color.

Several ions, including chromic, nickelous, and uranyl interfere because of the ion's color. In addition to their own color, the interference of copper and cobalt arises from the formation of colored complexes with thiocyanate. Curves for solutions containing these two ions are shown in Figure 3. These complexes are not stable in water. In this colored group the ceric ion inter-

Table II. Effect of Diverse Ions

Ion	Added	Ion Concn. *P. p. m.*	Iron Concn. *P. p. m.*[a]	Permissible Concn. *P. p. m.*[b]
Ag^+	$AgNO_3$	0	Ppts.	0
AsO_3^{+++}	Na_3AsO_3	500	1.96	500
Ba^{++}	$Ba(NO_3)_2$	20	2.00	20
Bi^{+++}	$Bi(NO_3)_3$	20	1.95	15
$B_4O_7^{--}$	$Na_2B_4O_7$	250 B_2O_3	1.94	150
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	50	1.94	30
Ca^{++}	$Ca(NO_3)_2$	200	2.00	200
Cd^{++}	$Cd(NO_3)_2$	500	1.95	400
Ce^{++++}	$Ce(SO_4)_2$	500	2.08	250
Co^{++}	$Co(NO_3)_2$	50	New hue	0
Cr^{+++}	$Cr_2(SO_4)_3$	50	New hue	50
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	50 Cr	New hue	50
Cu^{++}	$Cu(NO_3)_2$	10	New hue	0
F^-	NaF	500	1.95	400
Hg^{++}	$HgCl_2$	50	1.96	50
Hg^+	$Hg_2(NO_3)_2$	0	Ppts.	0
I^-	KI	50	1.64	5
MoO_4^{--}	$(NH_4)_2MoO_4$	50	New hue	0
Ni^{++}	$Ni(NO_3)_2$	50	New hue	50
NO_2^-	KNO_2	50	1.82	10
Pb^{++}	$Pb(C_2H_3O_2)_2$	10	2.01	10
HPO_4^{--}	$(NH_4)_2HPO_4$	500 P_2O_5	1.97	700
$P_2O_7^{----}$	$Na_4P_2O_7$	50	1.72	5
Sb^{+++}	$SbCl_3$	50	1.93	25
SO_3^{--}	Na_2SO_3	500	1.94	300
$S_2O_3^{--}$	$Na_2S_2O_3$	50	1.56	3
$SnCl_4^{--}$	H_2SnCl_4	50 Sn	1.96	50
$SnCl_6^{--}$	H_2SnCl_6	50 Sn	1.96	50
Sr^{++}	$Sr(NO_3)_2$	0	Ppts.	0
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	50	New hue	30
VO_3^-	$NaVO_3$	10	1.97	10
WO_4^{--}	Na_2WO_4	50	1.97	50
Zn^{++}	$Zn(NO_3)_2$	50	1.94	30
ZrO^{++}	$ZrO(NO_3)_2$	10 Zr	2.01	10

[a] Calculated from 2 p. p. m. used.
[b] To keep error within 2 per cent.



feres positively, the presence of 500 p. p. m. causing an error of 4 per cent.

Summarized information on the effect of interfering cations is given in Table II.

Anions. Of the 30 anions studied acetate, arsenate, benzoate, bromide, carbonate, chloride, citrate, cyanide, formate, nitrate, phosphate, salicylate, silicate, sulfate, and tartrate do not interfere more than 2 per cent in concentrations 250 times that of the iron.

Several ions, such as fluoride, oxalate, and pyrophosphate, form colorless complexes with the iron. Interestingly, the effect of fluoride is very marked in water solution but small in acetone. In order to keep the error within 2 per cent, the concentration must be less than 30 p. p. m. in water and 400 p. p. m. in acetone. Pyrophosphate should not exceed 5 p. p. m., and oxalate must be completely absent in water and not exceed 30 p. p. m. in acetone.

Chlorostannite, iodide, nitrite, sulfite, and thiosulfate interfere by reducing the iron. Arsenite, chlorostannate, and tetraborate have a small bleaching effect.

A change of hue is produced by vanadate and dichromate, and the latter oxidizes the reagent. Molybdate, on reduction to quinquivalent molybdenum, forms colored complexes with thiocyanate and must be absent.

Tungstate interferes because of the low solubility of tungstic acid.

Summarized information on the effect of interfering anions is given in Table II.

Permanent Standards

The permanent standards studied were designed to match the color obtained by following the A. P. H. A. procedure. This includes the use of 10 ml. of 2 per cent ammonium thiocyanate, 2 ml. of 3 *N* hydrochloric acid, and the required amount of iron diluted to 100 ml.

Solutions of three mixtures of salts were used: cobaltous chloride and potassium chloroplatinate, each in 2.4 *N* hydrochloric acid; cobaltous chloride and ferric chloride, in 1 per cent hydrochloric acid; and hexamminocobaltic nitrate and pentamminoaquocobaltic chloride, each in 2.8 per cent ammonia. Table I shows the composition of the solutions used to color match the standards which contained 2.0, 1.0, 0.5, and 0.2 p. p. m. of iron.

Visual comparison of the solutions was made in 24-cm. Nessler tubes and the spectrophotometric curves were determined with cells 4.98 cm. thick. The curves for 24-cm. thickness were calculated with the color slide rule from the data for 4.98 cm. Figure 4 shows curves for solutions containing 0.2 and 0.5 p. p. m. of iron, together with the corresponding standards, at a thickness of 4.98 and 24 cm. After the cobaltammine standards stood 110 days, the slight precipitate that developed was filtered off and the curves were redetermined. The standards had faded somewhat in this period. They cannot be recommended, therefore, for use longer than a month after preparation.

The calculated trichromatic coefficients and other colorimetric data for the iron thiocyanate and the different permanent standards are shown in Table III for a concentration of 0.5 p. p. m. of iron. Using the principle of least squares, it may be seen that the cobaltammine mixture is the best match for this concentration.

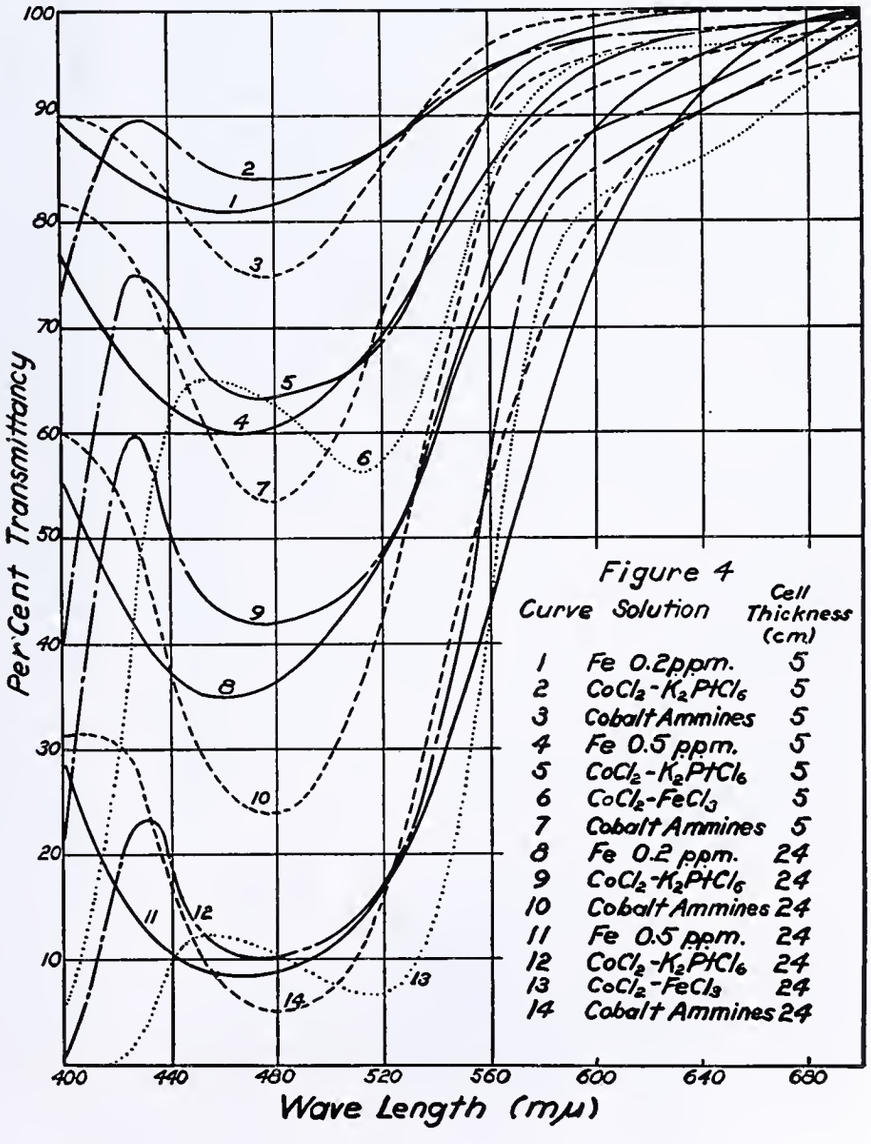


Table III. Colorimetric Values for Solutions of Iron and Thiocyanate (0.5 p. p. m.) and for Corresponding Color Standards

Solutions	Trichromatic Values: Red A[a]	Red B[b]	Green A	Green B	Violet A	Violet B	Least Square Sums[c] A	Least Square Sums[c] B
	%	%	%	%	%	%	%	%
$Fe^{+++} + SCN^-$	35.2	49.6	34.4	39.0	30.4	11.4	....	....
$CoCl_2$ / K_2PtCl_6	34.8	48.1	34.0	38.4	31.2	13.5	0.96	7.02
$CoCl_2$ / $FeCl_3$	36.4	51.0	32.8	38.2	30.8	10.8	4.16	2.96
$Co(NH_3)_6(NO_3)_3$ / $Co(NH_3)_5(H_2O)Cl_3$	35.3	48.4	34.4	39.3	30.3	12.3	0.02	2.34

Solutions	Monochromatic Values: Brightness A	Brightness B	Colorimetric Purity A	Colorimetric Purity B	Dominant Wave length A	Dominant Wave length B
	%	%	%	%	$M\mu$	$M\mu$
$Fe^{+++} + SCN^-$	81.9	44.0	19.0	69.5	583	590
$CoCl_2$ / K_2PtCl_6	85.1	51.1	16.5	64.0	584	590
$CoCl_2$ / $FeCl_3$	75.0	43.4	17.5	71.0	598	592
$Co(NH_3)_6(NO_3)_3$ / $Co(NH_3)_5(H_2O)Cl_3$	83.2	48.6	19.0	67.0	584	589

[a] Values for 4.98-cm. cell.
[b] Values for 24.0-cm. cell.
[c] Square of difference between values for iron and standard.

Discussion

In addition to seeking the best conditions for carrying out the thiocyanate method, this investigation was undertaken to evaluate the method in comparison with several other procedures which have been studied recently. Table IV presents comparative data for the following reagents: α,α'-bipyridyl, ferron (*19*), mercaptoacetic acid (*20*), *o*-phenanthroline (*6*), salicylaldoxime (*8*), salicylic acid (*11*), and thiocyanate. The thiocyanate method is inferior in several respects to several of the other methods. For general work *o*-phenanthroline is probably the best reagent in the list. Mercaptoacetic acid is recommended for total iron. Although either of these reagents is superior, in general, to thiocyanate, such conclusions may be subject to reservation. A given reagent might be best under some conditions but not under others.

In the determination of iron with thiocyanate the following variables must be kept reasonably constant: amount of reagent, amount and kind of acid, use of excess oxidizing agent, time of standing, presence and amount of certain interfering ions, and dielectric constant of the solvent. Since the intensity of the color formed depends upon the amount of reagent used, this factor must be carefully controlled. The amount and kind of acid, especially with hydrochloric or sulfuric, greatly affect the color intensity. With nitric acid the color is the same over the range 0.3 to 1.2 *N*, but there is some divergence from Beer's law. A pH range of 1.2 to 1.5 with this acid yields a more intense color which conforms to Beer's law, but the sensitivity to change in pH makes this impractical in analysis. Since the color fades about 10 per cent in 24 hours, matching must be made within 4 to 5 hours to keep the error less than 2 per cent. The use of acetone not only increases the sensitivity but also may decrease interference by some ions, especially fluoride; however, it introduces difficulties in case of salts which are somewhat insoluble in the acetone-water mixture.

Summary

By means of a spectrophotometer a critical study has been made of the colored system resulting from the interaction of ferric and thiocyanate ions. From several hundred spectral transmission curves obtained, of which those shown are representative, the following conclusions, relating to the use of this procedure for the colorimetric determination of iron, are evident:

Ammonium thiocyanate is preferable to thiocyanic acid as a color-forming reagent.

Nitric acid is preferable to sulfuric or hydrochloric acid.

With this acid Beer's law is followed through the pH range 1.2 to 1.5, but not in higher acidities.

Many of the 57 diverse ions studied interfere.

The sensitivity is increased approximately 100 per cent by using a 60 per cent acetone solution. This compound also improves the stability of the color.

A solution of two cobaltammine salts is an improved color standard except for a slight fading after several months.

In general, the thiocyanate method is inferior to several others, especially those using *o*-phenanthroline, α,α'-bipyridyl, or mercaptoacetic acid.

Table IV. Comparison of Reagents

Item Compared	Ferron	Mercapto-acetic Acid	Salicylic Acid	Salicyl-aldoxime
Fe concn. at 50% transmission[a]	4.5[b]	4.3[b]	10.1[b]	3.4[b]
Working range, 1-cm. cell	0.1–54[b]	0.1–14[b]	0.2–45[b]	0.1–22[b]
Stability	9 days	12 hours	65 hours	24 hours
pH range	2.7–3.1	7–12	2.5–2.7	6.2–6.6
Effect of pH change	Changes hue	None	Changes intensity	Changes hue
Color of reagent	Yellow	Colorless	Colorless	Colorless
Effect of excess reagent	Changes hue	None	Changes intensity	None
Conformity to Beer's law	None	Close	Close	Close

Item Compared	*o*-Phenan-throline	α,α'-Bi-pyridyl	Thiocyanate 60% aceton	Thiocyanate Water
Fe concn. at 50% transmission[a]	1.7[b]	2.0[b]	1.2[b]	2.4[b]
Working range, 1-cm. cell	0.05–6[b]	0.2–17[b]	0.05–5.0[b]	0.1–10[b]
Stability	6 months	6 months	4 hours	2 hours
pH range	2–9	3.5–8.5	0.3–1.2 *N*[c]	0.3–1.2 *N*[c]
Effect of pH change	None	None	Fades	Fades
Color of reagent	Colorless	Colorless	Colorless	Colorless
Effect of excess reagent	None	None	Changes intensity	Changes intensity
Conformity to Beer's law	Close	Close	Limited	Limited

[a] For 1-cm. cell and maximum absorption of curve.
[b] P. p. m. of iron.
[c] Nitric acid.

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Amyl Nitrite

A Method for Its Quantitative Determination and Some Observations on Its Decomposition

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LITTLE information is available in the literature in regard to the stability of amyl nitrite in the presence of light and air. The only authors who mentioned this specific property agreed that amyl nitrite was probably unstable in light and air. Tyndall (*14*) in 1869 used saturated amyl nitrite vapor to demonstrate a band of light now known as the "Tyndall beam", and concluded that amyl nitrite was rapidly decomposed by strong rays of light. Spiegel (*11*) added that amyl nitrite is also unstable in air. Leffmann (*5*) stated that amyl nitrite in air, probably influenced by light, decomposes. While investigating certain toxic factors of amyl nitrite, it was observed that the compound did not decompose in the presence of light and air as rapidly as reported in the above references. In order to obtain further information, the following work was undertaken.

For this study, a method suitable for detecting amyl nitrite in the vapor state was necessary. Since the authors were concerned primarily with its stability at low concentrations in air, a method that employed a less sensitive reagent with more color stability than those previously described (*1, 7, 13*) would permit sampling gas continuously over a long period of time. This eliminated the necessity of taking frequent readings or making dilutions. A reagent which was unaffected by light and heat was also desired. Because amyl nitrite is readily destroyed by strong acids (*5*), all methods employing such acids were unsatisfactory. Likewise, liquids in which amyl nitrite is freely miscible (*5*) could not be utilized for gas sampling because they decomposed the amyl nitrite, were too easily vaporized, or tended to interfere in the subsequent color reaction.

Liebermann (*6*) found that characteristic colors were produced when sodium nitrite was mixed with phenol and sulfuric acid. Later, Feigl (*4*) adopted this reaction for detecting qualitatively a number of organic nitrites. Ware (*17*) in a method which he considered specific for distinguishing phenol, the cresols, and certain other related phenolic compounds, utilized copper sulfate, acetic acid, and a minute quantity of sodium nitrite to produce a color. In following Ware's method, the authors found that when amyl nitrite was substituted for sodium nitrite, a similar color was produced. They subsequently adapted the method to the quantitative detection of amyl nitrite.

Method

The chemicals used were 5 per cent phenol solution made with reagent grade white phenol crystals, 2 per cent hydrated copper sulfate solution, and C. P. glacial acetic acid. The isoamyl nitrite, having a boiling point of 95–98° C. and an index of refraction at the D line and 20° C. of 1.3867, was prepared by C. S. Marvel (University of Illinois), following the method of Noyes (*9*) for preparing *n*-butyl nitrite.

When a minute amount of amyl nitrite, or any other nitrite, is added to a mixture consisting of phenol solution, copper sulfate solution, and glacial acetic acid, the color of the mixture changes from a faint blue to a deep red. This color reaches its maximum intensity within 15 minutes at 25° C., and if the solution is heated at 80° C. in a water bath only 5 minutes are necessary. The color remains stable for at least 24 hours.

The proportions of the solution were modified for the quantitative method in one respect. Since as much as 1 ml. of glacial acetic acid was found not to interfere with the development of the color, that quantity was used principally as a solvent for amyl nitrite. A stock reagent was prepared for use in the subsequent quantitative determinations by mixing phenol solution 24 parts, copper sulfate solution 1 part, and glacial acetic acids 5 parts. This reagent remained stable for at least 2 months.

Using a color of medium intensity, the spectral transmittance with a Coleman double monochromator spectrophotometer through a 5 mμ slit was determined, and the resulting curve is shown in Figure 1. Maximum absorption was obtained at 515 millimicrons. The shaded area indicates the range of a 520 mμ filter.

The test was performed with amyl nitrite, amyl alcohol, valeraldehyde, ethyl alcohol, oxalic acid, and crystals (probably ammonium tetraoxalate, *12*) formed by evaporating amyl nitrite, to determine whether or not impurities and decomposition products of amyl nitrite entered into the reaction. No colors were produced.

In preparing the calibration curve (Figure 2), standards were made by mixing 4.8 ml. of phenol solution, 0.2 ml. of copper sulfate solution, and 0.025 to 1.0 ml. of an accurately weighed quantity of amyl nitrite diluted with glacial acetic acid. Of the diluents available for amyl nitrite, glacial acetic acid was chosen because it does not interfere with the color reaction, whereas ethyl alcohol in amounts above 0.5 ml. reduces the color intensity and amyl alcohol is not miscible with the other reagents at room temperature. At no time was more than 1 ml. of glacial acetic

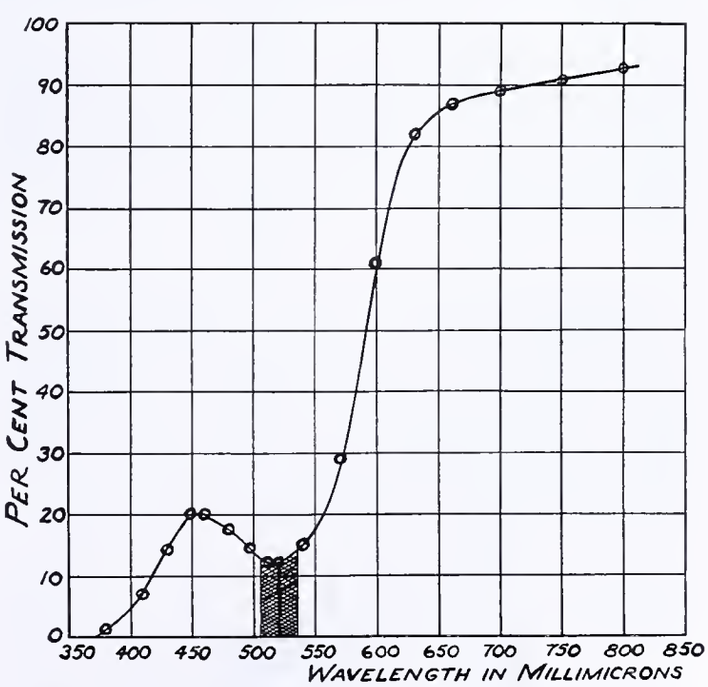

FIGURE 1. SPECTRAL TRANSMITTANCE CURVE FOR A COLOR OF MEDIUM INTENSITY

Shaded area indicates range of a 520 mμ filter.

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TABLE I. RESULTS OF SAMPLING METHODS

Sample No.	Calculated Concentration	Volume of Sample	Bubbling Rate	Found	Calculated Concentration
				P. p. m. by volume	
	P. p. m.	*Cc.*	*Cc./min.*		*%*
Samples taken using gas-collecting tubes and bottles					
A-1	1165	296	...	951	81.7
A-2	1165	291	...	1105	94.9
A-3	1165	283	...	1137	97.5
A-4	1165	296	...	1415	121.5
A-5	1165	291	...	1378	118.3
A-6	1165	283	...	1056	90.6
A-7	1165	296	...	1060	91.0
A-8	1165	296	...	1148	98.5
A-9	1165	291	...	1182	101.5
A-12	1165	323	...	1206	103.5
A-13	1165	291	...	1079	92.6
A-14	1165	296	...	1025	88.0
Av.	1165	..	...	1145	98.3
Average percentage deviation = 9.2					
R-9	506	283	...	578	115.0
R-10	506	250	...	461	90.2
B-2	506	291	...	483	95.5
B-3	506	274	...	427	84.4
B-4	506	296	...	552	108.7
S-1	506	600	...	431	85.2
S-2	506	330	...	548	108.3
Av.	506	..	...	497	98.2
Average percentage deviation = 11.0					
Continuous samples, sintered-glass absorbers					
A-E	1165	1700	425	1178	101.1
A-F	1165	1275	425	1260	108.1
A-G	1165	1275	425	1460	125.3
A-H	1165	420	51	1010	86.7
A-I	1165	675	56	1000	85.9
A-M	1165	445	8.7	1000	85.9
Av.	1165	..	...	1151	98.8
Average percentage deviation = 12.7					
B-A	506	1000	95	559	110.5
B-B	506	1000	133	439	86.8
B-C	506	1000	49	491	97.0
B-D	506	1000	66	586	115.8
B-E	506	1000	66	528	104.4
B-F	506	1000	42	533	105.3
B-G	506	1000	127	439	86.8
Av.	506	..	...	511	99.0
Average percentage deviation = 9.3					

acid used in the reaction. Volumes were made up to 6 ml. of glacial acetic acid when necessary, after which the tubes were heated in a water bath at 80° C. for 5 minutes. Readings were made on an Evelyn photoelectric colorimeter (*3*), using a 520 mμ filter. The color obeyed Beer's law within the 0 to 1800-microgram range.

By this method, 0.05 to 8 mg. of amyl nitrite per 6 ml. of reagent can be determined with accuracy. The accuracy of the method was unaffected when the colors were diluted with distilled water before making the colorimetric reading. Hence, when very strong colors were obtained, a portion was diluted with distilled water and the reading corrected for the amount of dilution.

Sampling of Air-Vapor Mixtures

To check the accuracy of the method, a weighed amount of amyl nitrite was placed inside a metal glass-lined chamber having a capacity of 5410 liters. Openings were sealed and light was excluded. An electric fan within the chamber circulated the air and mixed the vapor thoroughly. After the liquid had completely vaporized, samples were taken by two methods: continuous samples, and "grab samples". No more than 20 liters of a given concentration of gas was removed during any test; hence, the volume reduction was practically negligible—viz., 0.4 per cent.

CONTINUOUS SAMPLES. Thirty milliliters of reagent were measured into each of three sintered-glass absorbers (G-1 porosity), connected in series. These absorbers were of the type shown in (*2*). The absorber system was connected to the chamber, the outlet end to a Mariotte's aspiration bottle. The color reaction took place while the gas bubbled through the reagent, so that it was necessary to sample only until a desirable color had developed. Collected samples were allowed to stand 15 minutes to ensure maximum development of color. Six milliliters of solution were measured from each absorber and colorimetric readings were made. The total amount of amyl nitrite in the known volume of gas sampled was then computed, using the following formula:

$$\text{P. p. m.} = \frac{24.5}{117.09} \times \frac{\gamma}{V} \times \frac{10^6}{10^6} = 0.209\,\frac{\gamma}{V}$$

P. p. m. = parts per million by volume at room temperature (25° C.)
117.09 = molecular weight of $C_5H_{11}.ONO$
24.5 = molecular volume of gas at 25° C.
γ = micrograms of $C_5H_{11}.ONO$
V = volume of sample in liters

By determining the amyl nitrite separately in each of the three absorbers, and knowing the concentration sampled, it was possible to observe the efficiency of absorption. A check on the method was made against two known concentrations.

Room air was bubbled through the reagent for as long as 21 hours at 0.5 liter per minute without producing a detectable change in the color of the reagent. It was thus demonstrated that the presence of traces of nitrite in the room air would introduce no element of error in vapor samples.

The amount of amyl nitrite that can be detected by this colorimetric method when checked against a known concentration in a sealed tank is shown in Table I. The maximum error is ±20 per cent, but the average percentage deviation for all the samples is 10 per cent. The percentage of amyl nitrite collected in each of the three bubblers in series is noted in Table II. The first absorber collected about 70 per cent of the total amyl nitrite; the second, 20 per cent; and the third, 10 per cent; the percentage increasing slightly with a lowered sampling rate.

From results not included in Table II, the authors have found that a sintered absorber with an average opening size of about 5 microns (approximately G-3 porosity) would give efficiencies ranging from 93 to 97 per cent. This efficiency was obtained when sampling concentrations of 5 to 100 p. p. m. at rates of 40 ml. per minute or less with 30 ml. of reagent. This higher efficiency in collection is due to the greater dispersion and fineness of bubbles, since the liquid height was the same as the others. The latter type of unit was used for long-period samples (16 hours) from an animal-exposure chamber.

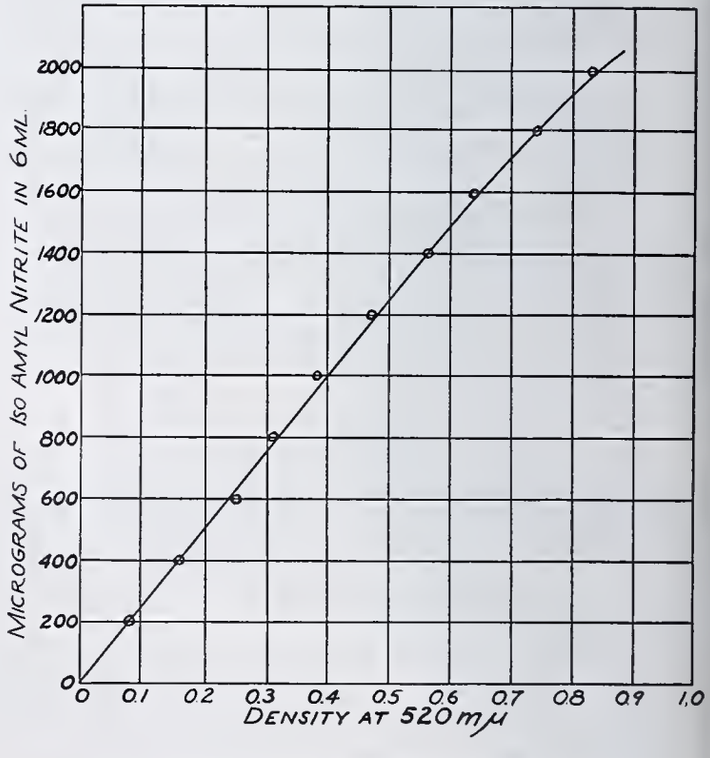

FIGURE 2. CALIBRATION CURVE

Ordinates are expressed in micrograms of isoamyl nitrite per 6 ml. Abscissas are based on 2-log G (G = galvanometer reading, *11*).

Table II. Absorption Rates and Efficiencies[a]

Rate	No. of Samples	Concentration	Retained per Absorber, 30 Cc. of Reagent in Each Absorber 1	2	3
Cc./min.		*R. p. m.*	%	%	%
425	3	1165	69.8	19.1	11.1
127	1	506	64.1	23.8	12.1
95	1	506	70.0	18.7	11.3
66	2	506	68.5	21.6	9.9
49	1	506	70.0	19.1	10.9
42	1	506	72.5	19.6	7.9

[a] Based on known concentration and obtained with sintered absorbers of 40–50 micron porosity.

Table III. Decomposition Rates of Air-Vapor Mixtures

(In unlighted tank samples taken by both grab and continuous methods)

Time of Exposure	Initial Concentration	Final Concentration	Remaining
Hours	*P. p. m.*	*P. p. m.*	%
0	1165	1165	...
2	1165	1178	...
6	1165	1201	...
25	1165	1165	...
27	1165	1206	...
48	1165	1053	...
21 days[a]	1165	1203	...

[a] Grab sample taken from tank on third day and kept in dark room.

Grab Samples. The vessels used were Pyrex gas burets with a capacity of about 300 ml., having ground-glass stopcocks at each end. The sampling tubes were evacuated before sampling and when taking the gas specimen were also flushed by means of a hand-aspirating bulb. To the amyl nitrite in the grab-sample tube, 6 ml. of reagent were pipetted in through one of the stopcocks by means of a capillary funnel which extended well down into the flask (*16*). After the closed vessel had been shaken for 15 minutes, the solution was drained into a colorimeter tube and read.

Decomposition of Amyl Nitrite

Stability of Amyl Nitrite in Air. From the large tank, sealed and dark, containing amyl nitrite vapor at a concentration of 1165 p. p. m., gas samples were taken with the bubbler system, first at half-hour intervals, then hourly, and finally only occasionally, for a period totaling 48 hours. Grab samples were also obtained, stored in a dark cool place, and analyzed 3 weeks later. Amyl nitrite in a sealed dark tank showed no decomposition in 48 hours (Table III). The concentration in grab-sample bottles stored in a dark cool place did not decrease significantly in 21 days.

The effect of artificial light on amyl nitrite vapor was observed by the same procedure. (Light measurements were made with a General Electric foot-candle meter.) In the large chamber, a concentration of 506 p. p. m. of amyl nitrite vapor was prepared. After control samples were taken, the inside of the tank was illuminated (average illumination 25 foot-candles), and the concentration was measured every half hour for 3 hours. There was little or no decomposition at the end of that time. To observe the effect of artificial light which he reported, the experiment described by Tyndall (*14*) was reproduced as closely as possible. (Illumination used was approximately 200 foot-candles.) His statement was unconfirmed, since the amyl nitrite remained unaffected even after the appearance of a Tyndall beam, which did not develop in the saturated vapor at room temperature and pressure until after 7 minutes' continuous exposure.

Influence of Diffuse Daylight on Vapor. Ground-glass stoppered bottles having capacities of from 250 to 600 ml. and 300-ml. gas-sampling tubes were filled with amyl nitrite vapor obtained from the large tank containing a concentration of 506 p. p. m. The bottles were filled in the manner described by Viles (*16*) and were placed where they would be exposed to the average daylight of a room (6 to 8 foot-candles). (The direct rays of the sun never entered the room because its only window faced directly north.) For controls, brown bottles were filled at the same time and placed beside the other bottles. A bottle was withdrawn at 24- to 48-hour intervals and the amyl nitrite content determined. The rate of decomposition when exposed to average daylight indoors is given in Table III. Amyl nitrite was still found in the clear bottles up to 12 days; in the brown bottles there was no decrease from the original concentration.

The experiment was repeated by placing bottles filled with vapor out of doors on a clear day (7000 to 10,000 foot-candles) and again when the sky was overcast (1000 to 2000 foot-candles). Several brown bottles were included as controls. Every 30 minutes a set of four was removed for analysis. After exposure to sunlight, only 20 per cent was recovered at the end of 1 hour. Within 2 hours, decomposition was complete (Table III). On a cloudy day, complete decomposition was obtained in 3 hours of exposure (Table III).

It has been intimated that decomposition in light is lessened if amyl nitrite is in a liquid state (*11, 14*). To study the effect of such a condition, 30 drops of isoamyl nitrite were diluted with 100 ml. of isoamyl alcohol in a brown glass-stoppered bottle and the amount of amyl nitrite per milliliter was determined. A 25-ml. glass-stoppered Pyrex flask was filled with this solution and placed in the direct sunlight. At half-hour intervals, 0.2 ml. was removed and mixed with 6 ml. of reagent. Amyl alcohol was not soluble in the reagent at room temperature, but when the mixture was heated in a water bath at 80° C. a clear solution was obtained. The colorimetric reading was made before the liquid cooled. Samples were taken and analyzed as long as significant change took place in the concentration. It was found that decomposition of liquid took place at almost the same rate as the vapor (Table IV). A Pyrex flask, similarly filled, was placed in a photographic darkroom and exposed to illumination placed 30 cm. (12 inches) away (approximately 70 foot-candles). Samples were taken at half-hour intervals for the first 3 hours, and then again after 26 hours of continuous exposure to the light. At that time, it was found that there was a 15 per cent decrease in the concentration (Table IV).

Discussion

The mechanism of the reaction is not known. Liebermann (*6*) and Feigl (*4*) state that they can offer no explanation. Manchot (*8*) suggests that the violet coloration produced by nitric oxide in solutions of copper sulfate in concentrated sulfuric acid is due to the formation of an easily dissociated compound, $CuSO_4NO$. It may be that copper sulfate acts as a catalyst in producing a diazo compound.

Ethyl alcohol is used as the diluent in the method for assaying amyl nitrite described in the 11th revision of the U. S.

Table IV. Decomposition of Amyl Nitrite Diluted with Amyl Alcohol

Time of Exposure	Initial Concentration	Final Concentration	Remaining
Hours	*Mg./liter*	*Mg./liter*	%
Exposed to direct sunlight in Pyrex flasks (7000 to 10,000 foot-candles)			
0	1740	1740	100
0.5	1740	1260	72.4
1	1740	750	43.1
1.5	1740	525	30.2
2	1740	215	12.3
2.5	1740	185	10.6
3	1740	125	7.2
Exposed to 50-watt light in reflector at 30-cm. distance (70 foot-candles)			
0	1740	1740	100
0.5	1740	1700	97.7
1	1740	1650	94.8
2	1740	1640	94.2
3	1740	1625	93.4
26.5	1740	1500	85.2
In animal exposure room (6 to 8 foot-candles), soft-glass bottle samples			
0	506	519	102.5
17.75	506	481	95.1
41.75	506	529	104.5
90	506	400	79.0
138	506	158	31.2
234.5	506	62.3	12.3
282	506	25	4.9
330	506	0	0
In outside daylight (1000 to 2000 foot-candles), Pyrex, sampling bottles			
0	506	504	99.7
0.5	506	509	100.5
1	506	317	62.6
1.5	506	219	43.3
2	506	77.5	15.3
2.5	506	30	5.9
3	506	0	0
In direct sunlight (7000 to 10,000 foot-candles)			
0	1165	1138	97.7
1	1165	195	16.7
2	1165	0	0

Pharmacopoeia (*15*) and it is recommended that the prepared solution be used within 0.5 hour. Amyl nitrite dissolved in amyl alcohol or ethyl alcohol was found to remain stable for at least a week when the solution was prepared under artificial light and stored in a brown bottle.

It is possible that amyl nitrite is decomposed by light of a specific wave length. Preliminary observations with ultraviolet and infrared light were made, but further investigation is necessary.

Conclusions

A method for the quantitative determination of amyl nitrite, adaptable for vapor-air and liquid analysis, is based on the fact that a color is produced when a compound containing an —ONO radical is combined with phenol and copper sulfate in acid solution. The method will detect amyl nitrite in air within an average deviation of 10 per cent. The advantage of this method is that the amyl nitrite vapor can be absorbed directly in the color-producing reagent. This eliminates the need for a separate solvent for amyl nitrite and simplifies the procedure because the reagent from the sampling vessel can be poured directly into a colorimeter tube and read; when determining unknown concentrations, the development of a color in the sampling tube indicates that nitrite is present and gages the length of time required to collect an adequate sample; and although the test does not compare in sensitivity with many of those already described (*1*, *5*, *7*, *10*, *13*), it has certain advantages for sampling a large volume of vapor at a low concentration for long periods. Amyl nitrite has been determined at concentrations of 5, 10, 20, and 100 p. p. m.

Amyl nitrite is stable in air and relatively stable when exposed to artificial light, but decomposes within 2 hours when exposed to direct sunlight.

Acknowledgment

The authors wish to express their appreciation to Otto Schales for determining the spectral transmittance of the color and for his many helpful suggestions.

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Cerate Oxidimetry

Determination of Glycerol

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GLYCEROL is ordinarily determined by oxidation, using an excess of standard potassium dichromate in sulfuric acid solution. The reaction, $3C_3H_8O_3 + 7K_2Cr_2O_7 + 28H_2SO_4 = 9CO_2 + 7Cr_2(SO_4)_3 + 7K_2SO_4 + 40H_2O$, requires an excess of dichromate and heating for 2 hours at 90° to 100° C. to complete the oxidation. The dichromate required for oxidation of the glycerol is determined by titrating the excess, using ferrous sulfate solution. For the back-titration the sulfuric acid concentration must be approximately 4 formal. The green color of the solution during the titration of excess dichromate makes necessary either an outside indicator "spot plate" ferricyanide estimation of the equivalence point or a potentiometric titration. The chief objection to the determination of glycerol by such procedure is the time required for the oxidation.

The present work describes a procedure by means of which this required time is reduced from 2 hours to only 15 minutes and the temperature from 90–100° to 50° C. The reaction upon which the method depends is $C_3H_8O_3 + 8H_2Ce(ClO_4)_6 + 3H_2O = 3HCOOH + 8Ce(ClO_4)_3 + 24HClO_4$. The excess of perchlorato ceric acid is then determined by titration, using standard sodium oxalate with nitro-ferroin (nitro-*o*-phenanthroline ferrous complex) as internal indicator. For this titration a 2 formal perchloric acid concentration is preferred. Oxidation by dichromate requires 14 equivalents per gram molecule of glycerol and by perchlorate cerate ion 8 equivalents.

Previous Work

The determination of glycerol by the dichromate procedure, using a potentiometric end point and the platinum-tungsten bimetallic electrode pair, has been described by the Chemical Division, Procter and Gamble Company (*6*). The same reaction using the same electrode system and an electronically operated automatic buret stopping device, was used by Shenk and Fenwick (*7*). Stamm (*13*) determined glycerol, using excess permanganate in strongly alkaline solution with titration of excess permanganate after acidification by use of oxalic acid. Glycerol was determined by Malaprade (*5*), using periodic acid in excess, followed by titration of the excess oxidant by one of several methods. By this procedure glycerol is oxidized to two molecules of formic acid and one molecule of formaldehyde. This oxidation of glycerol differs from the perchlorato cerate oxidation in that the formation of formaldehyde as an end product is eliminated in the latter case. The Malaprade reaction has been further studied by Allen, Charbonnier, and Coleman (*1*). Using the sulfato cerate ion, the oxidation of organic compounds in general results in the formation of formic acid as shown by Willard and Young (*14*), Cuthill and Atkins (*2*), and Fulmer, Hickey, and Underkofler (*4*).

The previous papers on cerate oxidimetry by Smith and coworkers (*3*, *8–12*) should be consulted for information con-

Table I. Oxidation of Formic Acid Using Perchlorato Cerate Ion

Time	Ce^{++++} N	0.1032 N Oxalate	HCOOH M	K Calcd.
Min.		*Ml.*		
		Temperature 51° C.		
0	0.0911	22.22	0.2254	.....
67	0.0846	20.47	0.2220	1.13×10^{-3}
157	0.0761	18.42	0.2178	1.16×10^{-3}
		Temperature 66° C.		
0	0.0914	22.30	0.2254	.....
73	0.0607	15.13	0.2100	5.6×10^{-3}
156	0.0407	9.85	0.2000	5.3×10^{-3}
240	0.0281	6.80	0.1939	5.3×10^{-3}

cerning preparation of reagents, preparation and stability of solutions, reaction potentials, and specific applications.

Rate of Reaction between Formic Acid and Perchlorato Cerate and Sulfato Cerate Ions

Since the oxidation of glycerol by the perchlorato or sulfato cerate ion involves the formation of formic acid which might be expected to exhibit some reduction action of measurable rate, it became necessary first to study the influence of such possible effects. It is particularly appropriate to do this, since the oxidation of glycerol takes place over a short time interval in contact with excess oxidant at slightly elevated temperatures.

The rate of oxidation of formic acid by perchlorato cerate ion was measured by titrating at given time intervals 25-ml. aliquot portions of a solution of a known original cerate and formic acid concentrations maintained at constant temperature. The data are shown in Table I.

The second order reaction equation is:

$$K = \frac{2.3}{T(nc - a)} \log \frac{(c - x)a}{(a - nx)c}$$

where T is the time, a is the original concentration of tetravalent cerium, c is the original concentration of formic acid in moles per liter, x is the number of moles of formic acid oxidized by time T, and n is the oxygen equivalent of formic acid. Since the reaction is $2Ce^{++++} + HCOOH = CO_2 + 2Ce^{+++} + 2H^+$, we know that $n = 2$. From the constants obtained at the two different constant temperatures k as a function of T may be calculated from the Arrhenius equation

$$\log k_2/k_1 = A(1/T_1 - 1/T_2)$$

By such calculation A was found to be 1.1×10^4. Knowing the practical concentrations involved in the case of an actual determination of glycerol, it is possible to calculate the percentage of perchlorato cerate ion involved in the oxidation of formic acid. These working concentrations are approximately 0.03 molar for both the cerate ion and the formic acid. The calculated error per determination of glycerol is less than 0.05 per cent. However, since k_T increases rather rapidly with increase in temperature, the reaction temperature employed must be kept below a definite maximum, not above 60° C. and preferably at approximately 50° C.

The oxidation of formic acid by the sulfato cerate ion, $Ce(SO_4)_3^{--}$, in sulfuric acid solution was found to be so slow that it is impossible of measurement without resorting to absurd reaction concentrations or extended periods of time. This might be expected when one considers the wide difference in oxidation potential exerted by the sulfato cerate and perchlorato cerate ions under the conditions outlined (*9*).

Preparation of Reagents

Ammonium Perchlorato Cerate. Dissolve 55 to 56 grams of $(NH_4)_2Ce(NO_3)_6$ by adding the salt to a liter beaker containing 340 to 345 ml. of 72 per cent perchloric acid. Stir well during a half-minute interval and add 100 ml. of water. Again stir well for a half-minute interval and add an additional 100 ml. of water. Continue this procedure until the volume attains 1 liter. Transfer to a suitable glass-stoppered reagent bottle and store in the dark. It is preferable to store the perchlorato cerate solution in a black bottle, conveniently made by completely covering a reagent bottle with black electrical insulating tape. Such a solution requires standardization about every 15 days. Standardization is best carried out by use of a standard 0.1 N sodium oxalate solution according to the procedure described by Smith and Getz (*10*), using nitro-ferroin as indicator. Carbon or dust if allowed to come in contact with perchlorato cerate solutions catalyze their slight deterioration rate.

Sulfato Cerate Solution. Dissolve 63 to 65 grams of $(NH_4)_4Ce(SO_4)_4.2H_2O$ in 1 liter of 0.5 molar sulfuric acid. This solution is stable in storage and need not be kept in the dark.

Sodium Oxalate in 0.1 N Perchloric Acid. Dissolve 13.412 grams of Bureau of Standards standard of reference sodium oxalate, per liter of 0.1 formal perchloric acid. This solution is stable upon storage. It is used to standardize the ammonium perchlorato cerate solution and for titrating the excess of perchlorato cerate in the determination of glycerol. Nitroferroin is used as internal indicator in both cases.

Ferrous Sulfate. Dissolve 40 grams of Mohr's salt, $FeSO_4.(NH_4)_2SO_4.6H_2O$, per liter of 0.5 N sulfuric acid. Store this solution under hydrogen and standardize, using the perchlorato cerate solution with ferroin as indicator.

Ferroin and Nitro-Ferroin. The ferrous sulfate complex of *o*-phenanthroline (ferroin) and of nitro-*o*-phenanthroline (nitroferroin) are now reagents of commerce prepared in solution form ready for use in analysis.

Glycerol Treated Soap Lyes, and Soap-Lye Crudes. These samples together with analyses were supplied through the courtesy of the Colgate-Palmolive-Peet Co., Jersey City, N. J. Appreciation is hereby expressed for the courtesy thus extended.

Basic Lead Acetate. Dissolve 100 grams of pure lead acetate in approximately 750 ml. of water in a 1.5-liter Erlenmeyer flask. Add 100 grams of lead oxide (PbO) and boil for 1 hour, using the reflux condenser. Filter the solution thus obtained while still hot. Disregard cloudiness in the filtrate and store in a suitable container.

0.5 N Potassium Hydroxide in Alcohol. Dissolve 16 grams of pure potassium hydroxide in the least required amount of water. Add the aqueous solution to 1 liter of 95 per cent ethyl alcohol and mix thoroughly. Allow to age 2 or 3 days to permit any potassium carbonate thrown out of solution to settle.

Preparation of Sample for Glycerol Determination

Fats and Fatty Oils. Warm the sample on the steam bath until liquid at about 80° C., filter, allow the free water to separate out, and decant the supernatant liquid into a dry bottle. Weigh 10 grams of the prepared sample into a 250-ml. Erlenmeyer flask and add 100 ml. of 0.5 N alcoholic potassium hydroxide solution and several pieces of porous plate to prevent lumping. Boil the contents of the flask under a reflux condenser for 90 minutes. Transfer the solution to a 500-ml. beaker, rinse the flask thoroughly with hot water, add to the transfer beaker, and dilute to 350 ml. with water. Evaporate by careful boiling to approximately 50 ml., dilute to 250 ml., and re-evaporate to 50 ml.

Add 100 ml. of a solution of perchloric acid (20 ml. of 72 per cent perchloric acid diluted to 100 ml.) and heat on the steam bath until the fatty acids separate. Filter off the fatty acid cake and precipitated potassium perchlorate, washing the filter and precipitates with cold water. To the filtrate add sufficient basic lead acetate solution to precipitate any proteins which may be present. If no precipitate forms upon the first addition no more precipitant need be added; if a precipitate does form, avoid a large excess of reagent by stepwise precipitation. When proteins are completely precipitated, filter, concentrate the filtrate if necessary on the steam bath, transfer to a 500-ml. volumetric flask, and dilute to volume.

Repeat the procedure, omitting only the sample, to make a blank determination as a control.

Soap-Lye Crudes, Treated Sample Lyes, and Distilled Glycerol. Weigh sufficient sample to contain about 1 gram of glycerol, using a 400-ml. beaker in the transfer. Dilute to 250 ml. and test the solution for proteins with basic lead acetate.

Table II. Analysis of Synthetic Glycerol Solutions Using Perchlorato or Sulfato Cerate

Sample No.	0.1036 N $Ce(ClO_4)_6^{--}$	0.1032 N $C_2O_4^{--}$	Glycerol Present	Glycerol Found	Error in $C_3H_8O_3$
	Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*
1	25.00	3.27	25.88	25.92	+0.04
		3.28		25.91	+0.03
		3.29		25.90	+0.02
2	50.00	22.37	32.96	33.02	+0.06
		22.44		32.94	−0.02
		22.40		32.99	+0.03
	0.0767 N $Ce(SO_4)_3$	0.0547 N $FeSO_4$			
	Ml.	*Ml.*			
1	50	28.86	25.88	25.94	+0.06
		28.93		25.90	+0.02
		28.88		25.93	+0.05
2	50	17.77	32.96	32.93	−0.03
		17.71		32.97	+0.01
		17.64		33.01	+0.05

Table III. Determination of Glycerol in Palm Oil Using Perchlorato or Sulfato Cerate

(Sample 0.6732 gram. 10-ml. aliquot portions from 500-ml. dilution of glycerol. Average result from 10 aliquots following dichromate determination gave value 9.65 per cent glycerol)

0.1036 N $Ce(ClO_4)_6^{--}$	0.1032 N $C_2O_4^{--}$	C_2O_4 Blank	Glycerol Found	0.0767 N $Ce(SO_4)_3^{--}$	0.0547 N $FeSO_4$	$FeSO_4$ Blank	Glycerol Found
Ml.	*Ml.*	*Ml.*	%	*Ml.*	*Ml.*	*Ml.*	%
25.00	8.56	..	9.64	50.00	39.16	1.32	9.64
	8.59	0.75	9.66		38.99	1.35	9.60
	8.58	0.75	9.67		39.09	..	9.66
	8.59	..	9.66		39.12	..	9.65

Filter if necessary, transfer the filtrate to 500-ml. volumetric flask, and dilute to volume.

Procedure in Determination of Glycerol

This procedure is a modification of standard soap manufacturer's methods with minor modifications.

Transfer samples of 10.00-ml. aliquots of the prepared solution to 400-ml. beakers. Add from a pipet a measured portion of standard perchlorato cerate solution which is known to be a sufficient excess. Dilute to 100 ml. with 4 formal perchloric acid. Warm on the steam bath to 50° C. and allow the reaction to proceed for 15 minutes at this temperature. The reaction temperature should not be allowed to exceed 60°. Cool and titrate the excess of perchlorato cerate ion with standard sodium oxalate solution, using 2 drops of 0.025 M nitro-ferroin as indicator.

If for any reason the use of perchlorato cerate standard solutions and perchloric acid is objectionable the usual procedure (*6*) may be employed, in which case a sulfuric acid solution is substituted for the perchloric acid solution of the glyerol and the sulfato cerate solution in sulfuric acid is used as oxidant. The sample and excess oxidant must be heated to 90 to 100° C. for 90 minutes before back-titration, using standard ferrous sulfate to determine excess sulfato cerate, with ferroin (in place of nitro-ferroin) as indicator.

In this case the chief advantage of the proposed procedure is lost—namely, a lower reaction temperature and shorter reaction time—although the advantages of ceric-ion oxidation are obvious as compared to dichromate oxidation, since there is no interference from the green color of the chromic ion formed in the latter case. The determination of glycerol by use of dichromate and ferrous sulfate need not be carried out potentiometrically (*6*), since excess dichromate can be titrated with ferrous sulfate in 4 formal sulfuric acid solution, using ferroin as indicator. The reverse titration of ferrous sulfate by dichromate is not similarly satisfactory.

Calculation of Results

Glycerol compositions are calculated following the formulation:

$$\text{Per cent } C_3H_8O_3 = 92C(A - B)/80\,DE$$

where A = ml. of cerate solution taken, multiplied by its standard factor
B = ml. of back-titrant required, multiplied by its standard factor
C = standard dilution of sample, ml.
D = sample weight
E = ml. of aliquot taken

Results Obtained by New and Old Procedures

Both the previously described methods were applied to the determination of synthetic glycerol solutions, with the results shown in Table II.

The determination of glycerol from palm oil was carried out with the results shown in Table III.

The determination of glycerol from distilled glycerol was carried out with the results shown in Table IV.

Summary and Conclusions

The determination of glycerol by the use of the perchlorato cerate ion in excess in perchloric acid solution, followed by back-titration using standard sodium oxalate, has a number of material advantages over the familiar dichromate–ferrous sulfate procedure. The time required for oxidation of glycerol is reduced from 180 to 15 minutes and the reaction temperature required is reduced from 90–100° to 50–60° C. Colorless solutions during titration make available the use of an internal oxidation reduction indicator—namely, nitro-ferroin—and makes unnecessary the use of a potentiometric method.

The perchlorato cerate procedure in perchloric acid medium has been checked by the use of sulfuric acid solutions and sulfato cerate oxidation without much saving of time but retaining the other advantages over existing procedures.

The internal oxidation-reduction indicators used in these reactions are nitro-ferroin for the perchlorato cerate procedure and ferroin for the sulfato cerate scheme. The end points in all reactions are sharp and distinct, and the accuracy attained in all cases is believed to compare favorably with existing procedures. Samples of commercially supplied materials of a variety of types have been used to illustrate the method.

No hazardous reactions were encountered in the progress of this work.

Table IV. Determination of Glycerol Distillates Using Perchlorato or Sulfato Cerate

(Sample 2.4437 grams. 10-ml. aliquot portions from 100-ml. dilution of sample. Average result from 6 aliquots following dichromate procedure gave value 97.81 per cent glycerol)

0.1036 N $Ce(ClO_4)_6^{--}$	0.1032 N $C_2O_4^{--}$	Glycerol Found	0.0767 N $Ce(SO_4)_3^{--}$	0.0547 N $FeSO_4$	Glycerol Found
Ml.	*Ml.*	%	*Ml.*	*Ml.*	%
25.00	4.95	97.86	50.00	32.09	97.87
	4.95	97.86		32.07	97.92
	4.97	97.74		32.15	97.72
	4.94	97.92		32.05	97.97

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A Procedure for Volumetric Determination of Gold

By Means of Potassium Iodide and Arsenious Acid

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SEVERAL volumetric methods for gold have been described in the literature, none of which is as rapid and convenient as the one here presented. All involve a quantitative reduction from the auric state either to the aurous or to metallic gold, and all but one require the absence of nitric acid.

Nitric acid is removed in the manner recommended by Gooch and Morley (*2*). The solution is evaporated several times with hydrochloric acid saturated with chlorine. The latter prevents partial decomposition to aurous chloride. The free chlorine is removed by neutralizing the acid with ammonium hydroxide, boiling gently, acidifying slightly with hydrochloric acid, heating to dissolve the fulminating gold precipitate formed with ammonium hydroxide, and again adding ammonium hydroxide and acidifying.

The permanganate titration described by Jüptner (*3*) and French (*1*) involves precipitation of metallic gold from an auric chloride solution free from nitric acid with an excess of standard ferrous sulfate or other reducing agent.

$$AuCl_3 + 3FeSO_4 \longrightarrow Au + Fe_2(SO_4)_3 + FeCl_3 \qquad (1)$$

Digestion on a steam bath accompanies this step, and the excess reducing agent is then titrated with potassium permanganate. The method is not applicable for gold in the presence of organic matter oxidized by permanganate.

Peterson (*5*) suggested a method based on the reaction of auric chloride and potassium iodide whereby aurous iodide and free iodine are formed.

$$AuCl_3 + 3KI \longrightarrow AuI + I_2 + 3KCl \qquad (2)$$

In titrating the liberated iodine with sodium thiosulfate, however, he obtained results which were not satisfactory. Gooch and Morley (*2*) developed conditions whereby good results could be obtained on very small amounts of gold.

According to their procedure, the weakly acid auric chloride solution is treated with a slight excess of potassium iodide, sufficient to precipitate aurous iodide and then redissolve it as the iodide complex. The liberated iodine is titrated to colorless with sodium thiosulfate, and a back-titration to a faint color is performed with iodine, adding starch indicator if desired. Dilute standard solutions, 0.01 *N* or 0.001 *N*, for example, are used. When using such dilute iodine solutions, a small correction is made, equivalent to that amount of the iodine which may be added to a blank containing starch before a color is produced.

Lenher (*4*) investigated the use of sulfurous acid in the titration of auric chloride solutions. It causes reduction of the yellow auric chloride to colorless aurous chloride. Various salts prevent partial reduction to metallic gold during titration. Magnesium chloride or sodium chloride intensifies the yellow color of auric chloride. Potassium iodide or potassium bromide may also be added to produce a strong color. A wide range in the amount of free hydrochloric acid is permissible. Small or large amounts of gold may be determined in this way, but the sulfurous acid requires frequent restandardization.

Rupp (*7*) held that the difficulty in applying Gooch and Morley's method for higher concentrations of gold was caused by the instability of aurous iodide, which slowly dissociated into metallic gold and iodine. Lenher's method, when potassium iodide is added to the auric chloride solution, is similar to that of Gooch and Morley, but sulfurous acid is used instead of sodium thiosulfate. Lenher, however, obtained accurate results in determinations ranging from 0.0395 to 0.7301 gram of gold in a volume of 100 ml. Furthermore, he did not find a back-titration with iodine necessary. His results indicate that the main error is caused not by the instability of aurous iodide, but rather by a side reaction involving the aurous iodide complex and thiosulfate.

Like auric gold, copper, iron, and platinum metals in their higher valence states also react with potassium iodide and with other reducing agents. One might therefore expect interference from such elements in both Gooch and Morley's and Lenher's methods. Where potassium iodide is used, however, error from this cause may be reduced or eliminated in some cases by avoiding more than a slight excess of iodide and by keeping the acidity low.

Rupp (*7*) precipitated metallic gold from an auric chloride solution with an excess of standard arsenious acid.

$$3As_2O_3 + 4AuCl_3 + 6H_2O \longrightarrow 3As_2O_5 + 12HCl + 4Au \qquad (3)$$

In this step the solution is heated until the colloidal gold disappears and the gold precipitate settles. The free acid is then neutralized with sodium bicarbonate, and the excess arsenious acid is titrated with standard iodine, using starch indicator.

For microdeterminations, Szebellédy and Viczián (*9*) titrated auric chloride solutions, to which sodium bromide was added, directly with arsenious acid. A gelatin solution prevented partial reduction to metallic gold. The titration was performed very slowly, with an interval of 15 to 20 seconds following each drop, and the temperature was kept at 90° to 95° C.

Pollard's method (*6*) applies to gold directly in the diluted aqua regia solution. In principle, it involves a titration with arsenious acid, whereby auric gold is reduced to the metallic state. Reduction from the auric to the aurous state is catalyzed by potassium iodide and mercuric chloride. Potassium bromide accelerates reduction from the aurous state to metallic gold, and in addition, it forms complex bromoauric acid, the color of which is removed by titration with arsenious acid.

The procedure requires consideration of the following reactions in aqua regia: Concentrated hydrochloric and nitric acids form chlorine and nitrosyl chloride, slowly at room temperature, more rapidly when heated.

$$3HCl + HNO_3 \longrightarrow Cl_2 + NOCl + 2H_2O \qquad (4)$$

When an aqua regia solution is diluted with water, nitrosyl chloride and chlorine recombine to form hydrochloric acid and nitric acid.

$$NOCl + Cl_2 + 2H_2O \longrightarrow 3HCl + HNO_3 \qquad (5)$$

Before diluting there is always an excess of nitrosyl chloride, and after diluting the excess reacts with water to form nitrous acid and hydrochloric acid.

$$NOCl + H_2O \longrightarrow HNO_2 + HCl \qquad (6)$$

When gold dissolves in aqua regia, it goes entirely into the auric state.

$$Au + 4HCl + HNO_3 \longrightarrow HAuCl_4 + 2H_2O + NO \qquad (7)$$

After solution of the sample, Pollard's method requires removal of the dissolved gases by bubbling air through the liquid as the latter is permitted to cool. This is necessary to avoid serious error from partial reduction to aurous chloride on diluting, because of the excess nitrosyl chloride originally present in the concentrated acid. A solution containing urea and potassium bromide is then added. A trace of nitrous acid, which catalytically starts the reaction between nitric acid and hydrochloric acid, is removed by the urea, and resolution of precipitated gold is thereby prevented. The mixture is heated to about 40° C. and titration is performed with a solution containing arsenious oxide, mercuric chloride, a small amount of potassium iodide, and some free hydrochloric acid. Auric gold oxidizes iodide as the latter enters and reappears in the solution. Iodine is thereby liberated, only to be reduced back to iodide by the arsenious acid.

Ordinarily, in acid solution an equilibrium exists between arsenious acid and free iodine.

$$As_2O_3 + 2I_2 + 2H_2O \rightleftharpoons As_2O_5 + 4HI \qquad (8)$$

In the presence of excess mercuric chloride, however, the iodide ion concentration is kept very low because of formation of the complex, $HgCl_2.HgI_2$. This permits the reaction to go quantitatively to the right. At the same time, the concentration of iodide is too low to reduce such substances as copper, iron, and platinum from their higher valence states. Interference on account of reduction is removed in the case of all metals except thallium, palladium, and iridium.

Reduction of gold to the metallic state causes formation of a precipitate which tends to make the solution opaque. This difficulty is overcome by passing air through the cold solution for a few minutes and then allowing the precipitate to settle.

When the brown color of bromoauric acid disappears, a small amount of gold remains in solution. This is determined by adding indicator containing sodium fluoride and *o*-tolidine or *o*-dianisidine, and titrating with hydroquinone solution. By forming a colorless complex, the sodium fluoride prevents an interfering color with any iron that may be present.

Ruthenium interferes in Pollard's method by forming a strongly colored solution which makes it difficult or impossible to find the end point.

While this procedure is rapid and accurate, it would be somewhat more convenient if metallic gold did not precipitate and tend to make the solution opaque during titration. It would be preferable, also, if the titration could be performed with a single standard solution instead of with two. The iodometric method given below has advantages in these respects. The reliability of the procedure has been established by a number of experiments in which good results were obtained under widely varying conditions.

Preparation of Standard Arsenite

The arsenite may be prepared according to the directions given by Scott (*8*).

If 0.01 *N* solution is desired, dissolve 0.495 gram of pure arsenious oxide in a little 20 per cent sodium hydroxide. Neutralize the excess alkali with dilute sulfuric acid, using phenolphthalein indicator, until the solution is just decolorized. Add 500 ml. of distilled water containing about 25 grams of sodium bicarbonate. If a pink color develops, remove it with a few drops of weak sulfuric acid. Dilute the solution to 1000 ml. and standardize it against a weighed amount of pure gold. The arsenite retains its strength practically indefinitely.

Recommended Procedure

If the gold content of a solution is to be determined, take with a pipet a sample containing about 40 mg. of gold. (Assume this to be 20 ml. or less.) In a fume hood add 15 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid, and heat briefly. Remove flame and add 25 ml. of 5 per cent sodium hypochlorite. This will be accompanied by a mild effervescence of chlorine gas. Add 35 ml. of distilled water and boil gently for 10 minutes. Cool, and neutralize the acid with a saturated solution of sodium carbonate or with a solution of sodium hydroxide, testing by touching the stirring rod to the edge of litmus paper. Adjust to slightly acid with dilute hydrochloric acid. Add a saturated solution of sodium bicarbonate until the gold solution reacts blue to litmus. Dissolve a few grams of potassium iodide in some distilled water and add the iodide rapidly, with stirring, to the gold solution. Add a few grams of sodium bicarbonate to ensure the presence of a sufficient excess, and titrate the liberated iodine with standard 0.01 *N* arsenite. Add a few milliliters of starch indicator solution when the iodine color becomes faint. The end point is the complete disappearance of the starch-iodine color.

The same procedure is applied to a solid, such as a gold alloy, the accurately weighed out sample of which is heated in the aqua regia until it dissolves.

Experimental

The standard solutions were prepared in the manner previously described by dissolving 5.0063 grams of arsenic trioxide and making the solution to volume, 1000 ml. The normality, 0.1012 *N*, was checked by standardization against a weighed amount of pure iodine. By proper dilution of the more concentrated solution, 0.01012 *N* arsenite was prepared. On the basis of Equations 2 and 8, the latter equation, of course, going completely to the right, 1.000 ml. of the 0.01012 *N* arsenite was theoretically equivalent to 0.998 mg. of gold.

The samples in experiments I, II, V, VI, and VIII were pure gold. The sample in experiment III was taken with a 1.00-ml. pipet from a solution of potassium aurocyanide, $KAu(CN)_2$, known to contain 20.3 mg. of gold per ml. Since phosphate is often present in industrial gold-plating solutions, pure gold was taken in experiment IV and 0.3 ml. of phosphoric acid was added to see if the latter would interfere. To the pure gold sample of experiment VII were added 10 mg. each of copper, iron, silver nitrate, and nickel to test for interference.

Table I. Results Which Illustrate Accuracy of Procedure

Expt.	Gold Taken	Arsenite	Gold Found	Deviation
	Mg.	*Ml.*	*Mg.*	*Mg.*
I	18.9	18.81 (0.01 *N*)	18.77	−0.1
II	19.4	19.19 (0.01 *N*)	19.15	−0.2
III	20.3	20.33 (0.01 *N*)	20.29	0.0
IV	22.9	22.88 (0.01 *N*)	22.84	−0.1
V	34.7	34.82 (0.01 *N*)	34.75	+0.1
VI	44.2	44.25 (0.01 *N*)	44.16	0.0
VII	233.7	23.38 (0.1 *N*)	233.4	−0.3
VIII	410.2	41.00 (0.1 *N*)	409.2	−1.0

Discussion of Procedure

Instead of bubbling air through the aqua regia solution as in Pollard's method, the reducing gas formed according to Equation 4 is more conveniently removed by introducing excess chlorine, then diluting and boiling out the excess. Addition of sodium hypochlorite solution or potassium chlorate causes the desired liberation of chlorine.

$$NaOCl + 2HCl \longrightarrow NaCl + H_2O + Cl_2 \quad (9)$$

$$KClO_3 + 6HCl \longrightarrow KCl + 3H_2O + 3Cl_2 \quad (10)$$

Effervescence attending the use of potassium chlorate is excessively rapid in warm, concentrated acid and mild in acid to which an equal volume of water has been added. If potassium chlorate is used instead of sodium hypochlorite, the recommended procedure is altered as follows: Add 1.0 gram of the material, heat the solution briefly, allow it to stand about 5 minutes while effervescing, dilute with 60 ml. of water, boil gently 10 minutes, etc. The excess chlorine is removed readily from the diluted solution by gentle boiling.

Neutralization of the acid does not cause precipitation of gold as a hydroxide or a carbonate. Upon addition of potassium iodide to the bicarbonate solution, the gold is reduced from the auric to the aurous state and iodine is liberated simultaneously according to Equation 2. The aurous gold remains in solution as the iodide complex.

The solution of auric gold treated with sodium bicarbonate is capable of oxidizing free iodine. This fact was discovered when a small amount of iodine solution or potassium iodide was added and the expected color with starch failed to appear. If potassium iodide solution is added rapidly, in large excess, and with stirring, no appreciable error occurs.

The titration of liberated iodine with sodium arsenite is represented by Equation 8, which goes quantitatively to the right in bicarbonate solution. The reaction is rapid, the end point is sharp, and starch may be used or omitted, as preferred. There is no tendency for excess arsenite to precipitate metallic gold.

Copper, iron, and other base metals when present do not interfere by reacting with potassium iodide. In the bicarbonate solution they form basic carbonate precipitates. Those which are colored tend to interfere by making it less easy to determine the end point. Platinum and palladium do not precipitate and do not cause liberation of iodine, but they form strongly colored iodide complexes which make the end point difficult to determine. The color, similar to that of free iodine in potassium iodide solution, is removed by arsenite only when a large excess is added.

Summary

Gold in an aqua regia solution is determined very conveniently by adding sodium hypochlorite or potassium chlorate, diluting, and boiling out the free chlorine formed with these substances. The acid is then neutralized with an excess of sodium bicarbonate, potassium iodide is added, and the liberated iodine is titrated with standard sodium arsenite, using starch if desired.

Acknowledgment

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An Improved Tangentimeter

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IN PERFORMING calculations with data from drying and filtration experiments, computing partial molal heat contents, partial heats of solution, and the like, it is frequently necessary to differentiate a function represented by a curve for which an equation is not easily obtainable, in order to obtain rates or instantaneous values at any desired point. Such differentiations are most conveniently performed by one of the well-known graphical methods, especially that involving the construction of a tangent to the curve and subsequently determining the slope of the tangent.

It has been recognized that the accurate location of tangent lines is attended with some little difficulty, particularly where the tangent is to be constructed to a line of small curvature, and that the errors resulting from such inaccurate construction may often be of considerable magnitude.

Various mechanical aids to the accurate construction of tangent lines have been used from time to time. Latishaw (*1*) described a simple tangentimeter in which a piece of speculum metal attached to a steel square was used to locate accurately the position of the tangent line. The chief disadvantage of this instrument was that it was usually necessary to draw in the tangent line with pencil or pen, then to calculate the slope. This drawing in of a large number of tangent lines, as is frequently required, may often result in some little confusion, as well as defacing the curve. In 1930, Richards and Roope (*2*) described a tangent meter which made use of a prism mounted on a vernier protractor scale, and this instrument was introduced commercially. Such an instrument, while accurate, made necessary the use of a factor to correct the observed tangent to the curve scale. In addition, it was somewhat expensive.

The tangentimeter here described is a modification of that described by Latishaw, being capable of a similar degree of accuracy. It possesses the additional advantages of not requiring the construction of any lines, thereby eliminating the possibility of confusion even when a large number of tangents are to be determined on a small portion of curve, and eliminating the necessity of defacing the curve that is being differentiated. Moreover, the instrument may be constructed very simply and inexpensively, is rugged, and may be made to any desired size, so that tangent intercepts may be read accurately and quickly from curves of large dimensions.

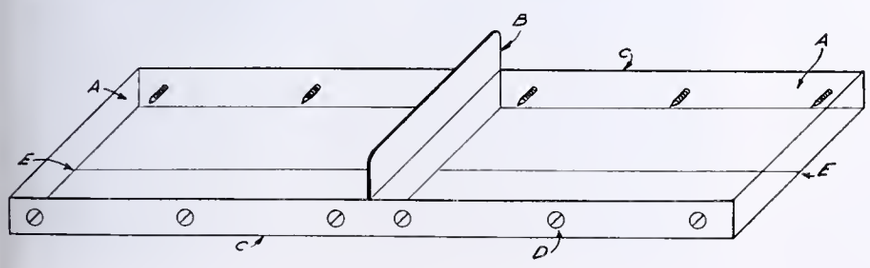

FIGURE 1. DETAILS OF CONSTRUCTION

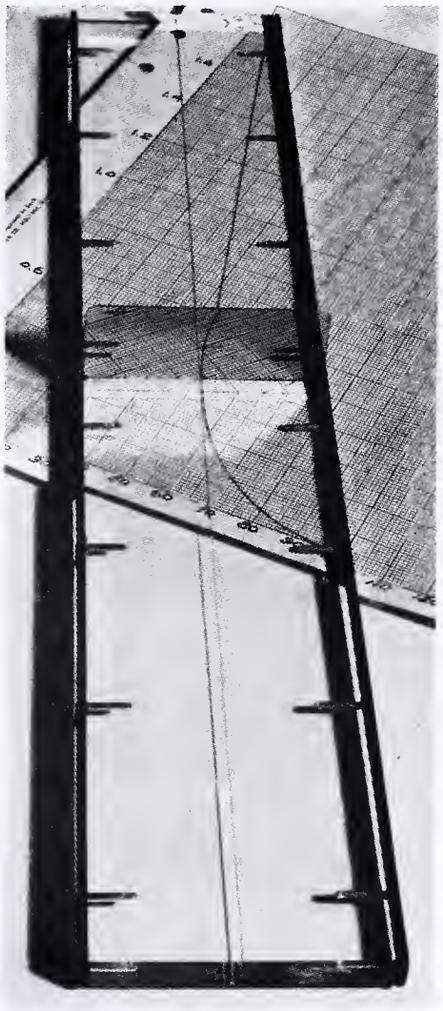

FIGURE 2. TANGENTIMETER IN USE

Figure 1 shows the details of construction of the tangentimeter. *A*, *A* are two pieces of transparent Lucite, 2 inches (5.0 cm.) wide and 0.375 inch (0.95 cm.) thick, and of any desired length. The ends of the plates should be highly polished to make them completely transparent. *B* is a metal mirror 2 inches (5.0 cm.) wide and 1 inch (2.54 cm.) high, which is set upright between the ends of the two Lucite plates and is soldered into shallow slots cut in the brass binding strips, *C*, *C*. These strips are 0.375 inch (0.95 cm.) wide and 0.06 inch (0.16 cm.) thick. The binding strips carrying the metal mirror are fastened to the plates by means of small machine screws, *D*, which fit into holes drilled and tapped in the Lucite plates. *E*, *E* is an index line scribed on the lower side of the plates, equidistant from the edges and exactly perpendicular to the metal mirror. The line may be easily scribed on the soft surface of the plastic by means of a machinist's scriber, a narrow engraving tool, or a sharp needle.

Figure 2 shows a photograph of the instrument in use. The tangentimeter is placed on the curve in such a position that the intersection of the index line and the mirror coincides with the point on the curve at which the tangent is to be located. The instrument is rotated about this point until the portion of the curve in front of the mirror and its reflection in the mirror appear as a continuous, smooth, unbroken line. When this position is obtained, it will be seen that the index line on the instrument is exactly tangent to the curve. The intercepts of the tangent line will be easily visible, and the slope may be very simply calculated.

This instrument has been used in this institution for the past year, both for research work and for student computations, with pronounced success.

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A Device for Starting the Swinging of a Balance

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SEVERAL methods are in use for starting the swinging of a balance in precise weighing. A current of air may be produced near one of the pans by a fanning motion of the hand, one of the pans may be pushed down by means of a fiber such as horsehair attached to a matchstick, or the beam may be given a push by means of a fiber attached to the rod carrying the rider. Guthrie (*3*) and Arzberger (*1*) have suggested that a puff of air be directed against the underside of one of the pans by squeezing a rubber bulb connected to a tube projecting through the base underneath the pan. None of these methods is altogether satisfactory, because the amplitude of the swing cannot be conveniently controlled. Furthermore, in the first two methods the balance case must be opened, and this is time-consuming and obviously unsatisfactory when hygroscopic objects are weighed.

The writer has used for some time a simple and convenient device which makes it possible to start the beam swinging without opening the case and to control the amplitude even while the beam is swinging. There are no references to previous use of the device except a rather obscure statement (*1*, *2*) in a work on balances. The present device makes use of the pull of a small electromagnet on a piece of soft iron or steel mounted on the beam or the pans of the balance. When the electromagnet is excited by closing a switch for a few seconds the pull exerted deflects the beam and starts it swinging.

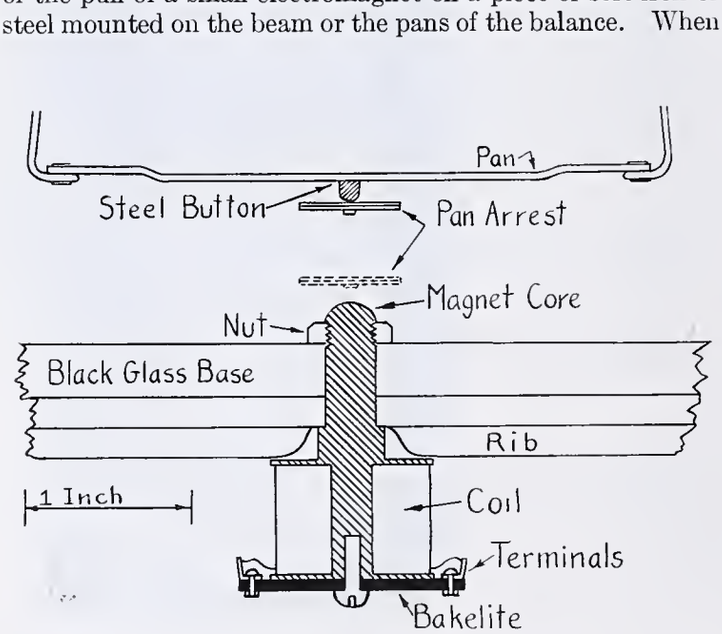

FIGURE 1. DETAILS OF ELECTROMAGNET

The balance used by the writer is a type T analytical balance made by Wm. Ainsworth & Sons and has a sensitivity of 0.05 mg. at a load of 200 grams. It was furnished by the maker with certain minor changes to permit the mounting of the device described below: a 0.79-cm. ($^5/_{16}$-inch) hole drilled in the black glass base just below the center of the left-hand pan for mounting the electromagnet, a 0.62-cm. (0.25-inch) hole in the front of the case for mounting a small push-button switch, and steel buttons on the underside of the pans in place of the aluminum buttons against which the pan arrests press in this make of balance. The steel buttons were plated to avoid possible corrosion.

In Figure 1 is shown the electromagnet mounted under the base of the balance and concentric with the center of the left pan, the core projecting through the hole in the base. A brass nut screwed on the threaded upper end of the magnet serves to hold it in place. The core of the electromagnet and the spool on which the exciting winding is wound are all in one piece turned out of cold-rolled steel bar. The spool is wound with about 350 turns of number 25 double cotton-covered magnet wire, impregnated with shellac and baked dry. The ends of the winding are soldered to lugs on a strip of Bakelite to facilitate connecting the coil into the exciting circuit. The presence of the stiffening rib indicated in Figure 1 necessitated making the magnet core longer than would otherwise have been necessary. The distance between the upper end of the core and the pan arrest was about 0.62 cm. (0.25-inch)—large enough to allow the pan arrest to be locked in the down position as indicated by the dotted lines of Figure 1. The drawer in the base had a notch cut out of the back end, to allow it to pass the coil which projects into the drawer compartment.

The push-button switch was made to operate with slight pressure and is shown in Figure 2. It consists of two strips, *A* and *B*, of 28-gage spring phosphor bronze 0.62-cm. (0.25-inch) wide with a contact point of copper on one of them. These springs are mounted on a strip of Bakelite which is clamped to the inside of the balance case by means of a nut that screws on the bushing in which the brass rod of the push button slides. A slight pressure on the push button pushes the two springs into electrical contact, thereby allowing the energizing current to flow through the electromagnet, as may be seen from the wiring diagram (Figure 3). The small Bakelite knob on the end of the push rod near the spring prevents the metal parts of the balance from coming into electrical contact with the circuit when the push button is operated. The electrical wiring is concealed in the space below the base and to the left of the drawer. A lamp cord passes through a small hole in the back of the balance and is connected to a 50-watt lamp serving as a current-limiting resistor and a plug for connecting to the 110-volt line. The most convenient place for the push button is to the left of the drawer.

The weighing is carried out in the usual way until balance is established closely enough to permit final determination of the

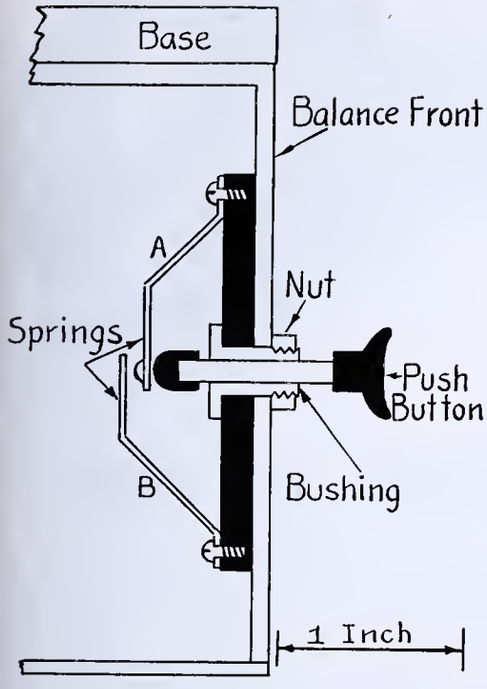

FIGURE 2. DETAILS OF PUSH-BUTTON SWITCH

point of equilibrium by the method of swings. With the balance closed, the beam is released and then the pans are released. To start the beam swinging the switch is closed long enough to give a deflection of 5 or 10 divisions. The switch is then opened and the beam allowed to swing freely. The amplitude of the deflection may be accurately adjusted by controlling the length of time the switch is kept closed. The beam can be brought to rest by closing the switch for a short time just as the pointer returns to zero from the side toward which the magnet deflects it.

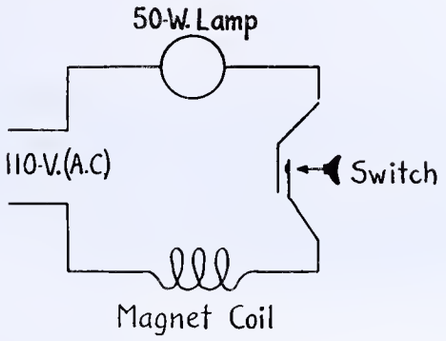

FIGURE 3. WIRING DIAGRAM

Careful tests with precision weights were made to determine possible errors that could be attributed to residual magnetism in the core of the electromagnet. This was done by weighing an object of accurately known weight first without the magnet in place and then with the magnet in place. No errors as great as the sensitivity of the balance (0.05 mg.) could be detected. However, errors could have been eliminated by using a solenoid without iron, in which case a somewhat longer coil with more turns of wire would have served as well.

The device may also be applied to other balances. For example, a deflecting force was applied to the steel pointer of an old balance by means of a small electromagnet mounted on the column of the balance by a clamping arrangement. If the small weight usually fastened to the pointer for adjusting the center of gravity is made of iron, a pull can be exerted on this by an electromagnet mounted on the column. Another possibility, not tried as yet, is to use a flat solenoid of rather large diameter mounted underneath one of the pans and fed with alternating current. This might be expected to result in a pull on the pan because of the eddy currents induced in it.

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CONTRIBUTIONS to Physics, No. 1.

Magnetic Stirrer for an Evacuated Sorption Apparatus

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IN ORDER to ensure that the vapor over a liquid is truly in equilibrium with the liquid, it is important to break the surface of the liquid continually, even in an evacuated system. Violent stirring is not desired because any liquid spray may cause a supersaturated condition.

The simple device shown in Figure 1 met these requirements admirably.

The glass bulb, *B*, containing the liquid to be stirred, has a heavy platinum wire support, *P*, sealed as shown into the wide neck of the bulb. This serves as the support for the stirring paddle, *S*, which is made from a glass rod flattened at the end. The upper part of the stirrer consists of a glass tube into which an iron nail, *N*, is sealed. The upper and lower parts of the stirrer are connected through a heavy platinum wire which is looped around the platinum support, *P*. The stirrer thus oscillates freely about *P* as an axis. *M* is a small electromagnet coil taken from an old electric doorbell. This is connected to the secondary coil of a doorbell transformer (18 volts). The 110-volt primary coil of the transformer is connected in series with a 20- to 40-watt lamp, in the socket of which is placed a 60-watt 120-volt "flasher button", a device for thermally making and breaking the circuit once or twice a second. The intermittent current through the primary of the transformer causes a similar intermittent current to flow through the secondary of the transformer and through the electromagnet. *N* is thus pulled over to *M* and released once or twice a second, causing *S* to oscillate slowly within the liquid.

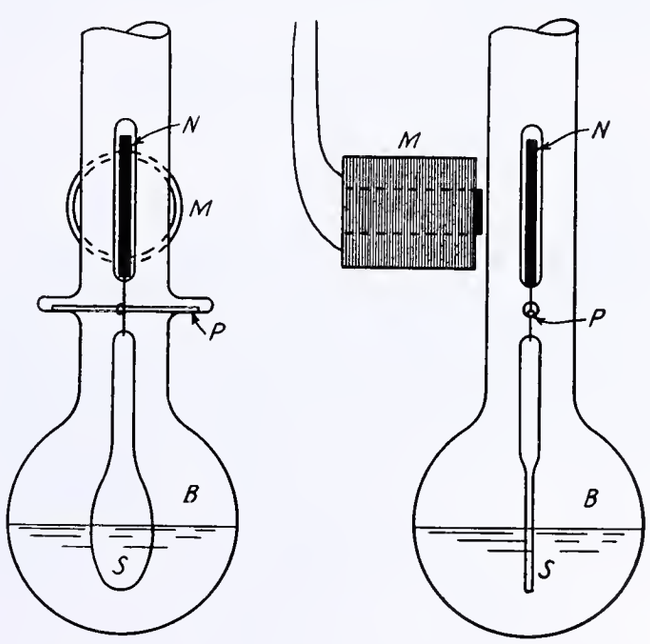

FIGURE 1. INTERNAL STIRRER FOR AN EVACUATED SORPTION APPARATUS

Microbiological Assay for Pantothenic Acid

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University of Wisconsin, Madison, Wis.

A convenient method of determining the pantothenic acid content of biological materials has been developed on the basis of the essential nature of this vitamin for a lactic acid bacterium. Crude suspensions of the sample are fermented by the organism, and the acid produced, as determined by direct titration of the entire culture, is a measure of the pantothenic acid in the sample.

The method gives results which agree well with values determined by chick assay, and is applicable to a wide range of natural materials. No expensive or unusual apparatus is required. One worker can assay approximately 15 samples per day.

THE bacterium *Lactobacillus casei* ϵ, which is used as the test organism in a microbiological assay procedure for riboflavin (*10*), also requires pantothenic acid[1] (*11*). A medium has been devised which is essentially free from pantothenic acid but appears to be otherwise adequate for *L. casei* ϵ. It has been found that on this medium the addition of graded amounts of synthetic calcium pantothenate results in a proportionate increase in lactic acid production. This relationship has been made the basis of a rapid assay method for pantothenic acid.

Cultures and Inoculum

The method of carrying cultures and preparing inoculum is indicated schematically in Figure 1.

The test organism, which may be obtained under the serial number 7469 from The American Type Culture Collection, Georgetown University Medical School, Washington, D. C., is carried as stab cultures in yeast water–glucose agar. This medium is prepared by dissolving 0.5 per cent glucose and 1.5 per cent agar in yeast water, which in turn is made by steaming fresh, starch-free baker's yeast for 2 hours with 10 times its weight of water, autoclaving 45 minutes at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure, allowing the cells to settle, and decanting the supernatant liquid. A 1 to 2 per cent solution of Difco yeast extract may be substituted for the yeast water if desired.

Sterilized tubes containing 10 cc. of this medium are inoculated with *L. casei* ϵ, incubated at 37° C. for 24 hours, and then stored in the refrigerator until needed. One such culture (Nos. 1, 2, etc.) is kept at all times as a reserve stock culture, which is never disturbed except to transfer at monthly intervals. At the time of this monthly transfer several extra stock cultures are prepared (Nos. 1a, 1b, 2a, 2b, etc.) and are used to grow inoculum for assay tubes as required during the month.

To grow inoculum a stab from a stock culture—e. g., 1a—is made into 10 cc. of the basal medium to which is added 0.5 microgram of calcium pantothenate. This culture, "subculture" in Figure 1, is incubated 24 hours at 37° C., and again transferred into the same medium. After 24 to 40 hours' incubation at 37° (but not longer) the cells are centrifuged out aseptically and resuspended in 10 cc. of sterile 0.9 per cent sodium chloride solution. One cubic centimeter of this suspension is then further diluted with 10 cc. of the sterile saline, and 1 drop of the final cell suspension is used to inoculate each assay tube. Thus cells

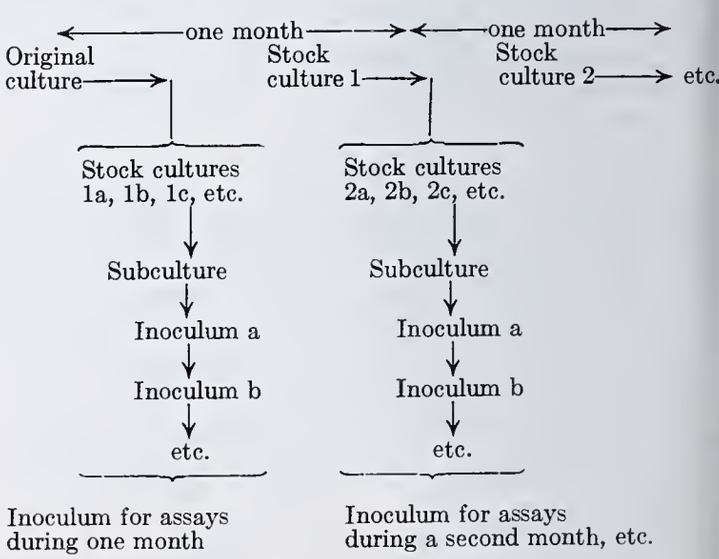

FIGURE 1. METHOD OF CARRYING CULTURES AND PREPARING INOCULUM

[1] In order to conform to recognized nomenclature (*1*), this organism will henceforth be termed *Lactobacillus helveticus* in publications from this laboratory.

from the agar medium pass through at least one subculture in the liquid medium before being used as inoculum.

If assays are to be made on each of several successive days, it is not necessary to grow inoculum from a different stock culture each day, but a drop from a tube of inoculum—e. g., a in Figure 1—may be transferred to a similar tube, b, which is incubated for use the next day. One should return to a stock culture about every 5 days, however, to minimize chances of contamination and bacterial variation.

Basal Medium

The basal medium contains sodium hydroxide–treated peptone 0.5 per cent, sodium acetate 0.6 per cent, glucose 1 per cent, cystine 0.01 per cent, asparagine 0.025 per cent, riboflavin 1.0 microgram per 10 cc. of medium, 0.05 cc. each of inorganic salt solutions A and B per 10 cc. of medium, and yeast supplement equivalent to 50 mg. of autolyzed yeast per 10 cc. of medium.

Sodium Hydroxide–Treated Peptone. To a solution of 40 grams of peptone (Difco Bacto) in 250 cc. of water are added 20 grams of sodium hydroxide also dissolved in 250 cc. of water. The mixture is allowed to stand at room temperature for 24 hours, the alkali is neutralized with glacial acetic acid (ca. 28 cc.), 7 grams of anhydrous sodium acetate are added, and the volume is made to 800 cc. with water. The final solution, which contains 5 per cent of peptone and 6 per cent of sodium acetate, is stored under toluene in the refrigerator.

Yeast Supplement. A solution of 25 grams of whole, autolyzed baker's yeast, obtainable from Difco Laboratories, Inc., Detroit, Mich., in 500 cc. of water is adjusted to pH 1.5 with concentrated hydrochloric acid, 25 grams of Norit A are added, the mixture is stirred 20 minutes at room temperature, and the charcoal is removed by either centrifuging or filtering with suction. In the latter case a 0.5-cm. layer of moistened Filter Cel (Johns-Manville, standard) is used on the paper. In either case the Norit is not washed, and a small amount of charcoal coming into the filtrate or centrifugate is disregarded. The pH of the liquid is readjusted to 1.5, and the adsorption repeated as before. The solution is then brought to neutrality with sodium hydroxide, filtered to remove any charcoal present, and diluted to 1000 cc. One cubic centimeter of the final solution is thus equivalent to 25 mg. of autolyzed yeast. It is desirable that the preparation of the yeast supplement be carried through as rapidly as convenient, so that the solution is not kept strongly acid for longer than 1 to 2 hours. This preparation is stored under toluene in the refrigerator.

Inorganic Salts. Solution A consists of 25 grams of dipotassium hydrogen phosphate and 25 grams of potassium dihydrogen phosphate dissolved in 250 cc. of water. Solution B consists of 10 grams of magnesium sulfate heptahydrate, 0.5 gram of sodium chloride, 0.5 gram of ferrous sulfate heptahydrate, and 0.5 gram of manganese sulfate tetrahydrate dissolved in 250 cc. of water.

Glucose. Only c. p. anhydrous glucose is used, and is added dry as needed.

Cystine. *l*-Cystine is dissolved in the minimum amount of hydrochloric acid, and the solution is diluted with water to a concentration of 0.1 per cent cystine. The solution is stored under toluene at room temperature.

Asparagine. A 1 per cent aqueous solution of *l*-asparagine monohydrate is prepared, and is stored under toluene.

Riboflavin. Merck's synthetic riboflavin is dissolved in 0.02 *N* acetic acid (dim light), and diluted with 0.02 *N* acetic acid to a concentration of 50 micrograms per cc. The solution is stored in the refrigerator under toluene and is carefully protected from light at all times.

Procedure

The assay cultures are grown in ordinary test tubes (16 × 150 mm. to 20 × 150 mm.) containing the basal medium plus the sample to be assayed in a total volume of 10 cc. A metal rack which holds each tube separately and upright and which can be autoclaved is very convenient but not necessary if the tubes are properly marked. If 50 assay tubes are to be set up from stock solutions of the above concentrations, 50 cc. of sodium hydroxide–treated peptone solution, 50 cc. of cystine solution, 12.5 cc. of asparagine solution, 5.0 grams of glucose, 1.0 cc. of riboflavin solution, 2.5 cc. each of inorganic salt solutions A and B, and 100 cc. of yeast supplement solution are mixed, adjusted to pH 6.8 to 7.0 with sodium hydroxide, and diluted to 250 cc. Five cubic centimeters are pipetted into each of the 50 tubes, and a suitable aliquot of the sample is added. The contents of each tube are then diluted to a total volume of 10 cc. Thus, as much as 5 cc. of liquid may be added with the sample. The volumes indicated should be measured with an accuracy of ±0.1 cc. The tubes are plugged with cotton, sterilized in the autoclave at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure for 20 minutes, allowed to cool, inoculated, and incubated at 37° C. (±1° C.) for 72 to 84 hours. The contents of the tubes are transferred to 125-cc. Erlenmeyer flasks, and titrated with 0.1 *N* sodium hydroxide to a definite pH of about 6.8 or 7.0. Bromothymol blue is a suitable indicator. A standard flask is used for color comparison.

Standard Curve

The range of the assay is roughly between 0.03 and 0.12 microgram of calcium pantothenate per 10 cc. of culture medium. Duplicate tubes should be set up at about the following concentrations of pure *d*-calcium pantothenate: 0.00, 0.025, 0.05, 0.075, 0.10, 0.125, and 0.15 microgram per 10 cc. of medium.

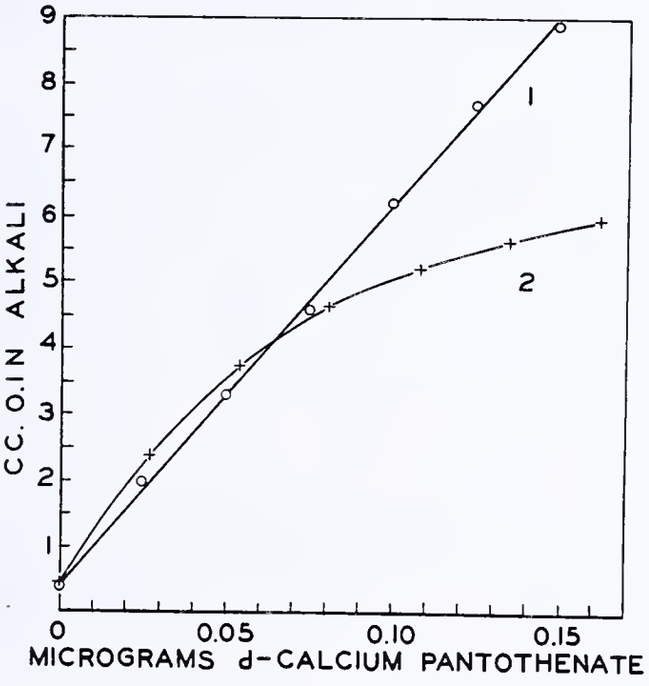

Figure 2. Standard Curve of Response to Pantothenic Acid

1. In presence of active yeast supplement
2. In absence of yeast supplement and asparagine

Acid production is plotted against calcium pantothenate concentration to obtain the standard curve (curve 1, Figure 2). This curve should be linear from the blank (1.0-cc. titration or less) very nearly to the maximum titration (8.5 to 9.5 cc.). Failure of the curve to meet these specifications is usually due to contamination or weakening of the test organism or to improper preparation of the yeast supplement. For comparison a typical "standard" curve as obtained on the basal medium without any asparagine or yeast supplement is included in Figure 2 (curve 2).

A standard curve is run with each individual set of assays. The pantothenic acid content of the unknown is determined in terms of calcium pantothenate by interpolation on the standard curve. Preferably, three levels which fall within the range 0.03 to 0.12 microgram should be run on each sample under test, with duplicate tubes at each level. The pantothenic acid content of the sample calculated from the various levels must agree within at least 20 per cent; otherwise the

results should not be trusted. The values at the three levels are averaged for the final results. The complete data for a typical analysis are given in Table I.

For the above purpose two stock solutions of pure *d*-calcium pantothenate (Merck, synthetic) in 0.05 *M* phosphate buffer (pH 6.8 to 7.0) are prepared. One is diluted to a concentration of 50 micrograms per cc., and the other to 5 micrograms per cc. The former is used as required to replenish the latter, which in turn is used from day to day in setting up assays. Aliquots are diluted accurately (with water) for setting up the standard curve. Both solutions are preserved under toluene in the refrigerator.

Preparation of Sample for Analysis

Solids are finely ground. Fresh tissues are mechanically homogenized in water. In either case a suspension is then prepared in approximately 50 volumes of water, adjusted to pH 6.5 to 7.5, autoclaved 20 minutes at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure, and neutralized to bromothymol blue (pH 6.8), and water is added to give a convenient dilution for assay. Aliquots of the final suspension are pipetted directly into the assay tubes.

Blood is diluted fivefold with water, and assayed directly without autoclaving. Other liquids such as milk or urine, and materials which are easily and completely soluble in water are simply diluted to a convenient volume, neutralized if necessary, and assayed directly without autoclaving.

Table I. Sample Calculation for Pantothenic Acid Assay

Preparation of Sample	Suspension Added per Tube	Titer, 0.1 *N* Base	Pantothenic Acid Content Per tube	Per cc. suspension	Per gram sample
	Cc.	*Cc.*	*Microgram*	*Microgram*	*Micrograms*
0.204 gram of dry yeast + 250 cc. of water, autoclaved, diluted to 500 cc.	2.0	4.5	0.067	0.34	83.4
	2.0	4.4	0.064	0.32	78.4
	2.5	5.0	0.078	0.31	76.0
	2.5	4.9	0.076	0.30	73.6
	3.0	6.0	0.100	0.33	80.9
	3.0	5.9	0.097	0.32	78.4
					Av. 78.4

Reliability of the Method

The reliability of the present method as a measure of pantothenic acid has been investigated, (1) by means of recovery experiments both on intact materials and on other materials from which the pantothenic acid was removed by autoclaving with strong acid or alkali, (2) by testing the effect of other known growth-promoting substances, and (3) by comparison with results secured on the same samples by chick assay. Data for typical recovery experiments are collected in Table II. The yeast extract (Difco) was hydrolyzed by autoclaving in *N* sodium hydroxide solution for 1 hour at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure. The liver extract was autoclaved in *N* sulfuric acid solution for 1.5 hours at the same pressure. Each sample was neutralized before assaying. The recoveries were satisfactory in all cases.

Attempts to carry out similar recovery experiments on whole tissues which had been hydrolyzed were in most cases unsuccessful. When beef liver or kidney was autoclaved for varying lengths of time up to 6 hours with *N* hydrochloric acid or *N* sodium hydroxide, and the neutralized product assayed, it was found that the bacteria did not grow normally in the assay tubes. Apparently some pantothenic acid remained intact in nearly every case, because a slight response was noted in the assay tubes containing the lowest level of the hydrolyzed samples. However, growth-inhibitory substances also seemed to be present, since in attempting to carry out the assay at several different levels in the usual manner, the tubes containing the larger amounts of sample actually showed less response than those containing smaller amounts. It was, therefore, impossible in such cases to determine how much pantothenic acid remained undestroyed, and reliable recovery experiments could not be carried out.

Table II. Recovery of Pantothenic Acid Added to Biological Materials

Sample	Pantothenic Acid Added	Pantothenic Acid Found	Recovery
	Micrograms/ gram	*Micrograms/ gram*	%
Dried yeast	None	22	
	20	43	105
Whole milk	None	4.0	
	5	8.4	88
Yellow corn	None	9.0	
	8.6	17.2	95
Wheat middlings	None	23	
	39	62	100
Rat kidney 1	None	16	
	28.8	47.3	115
Rat kidney 2	None	47	
	139	181	96
Human urine 1	None	2.25	
	1.67	3.93	101
Human urine 2	None	6.2	
	5.0	11.6	108
Beef liver	None	61.5	
	35.7	97.1	100
	71.5	129	94
Hydrolyzed yeast extract	None	None	
	125	137	110
Hydrolyzed liver extract	None	9.0	
	12.5	21.5	100

The effect of known growth-promoting substances was determined by assaying pure pantothenic acid in the presence of the following mixture of compounds (figures are micrograms in each culture tube): biotin 0.004, cozymase 0.8, indoleacetic acid 40, asparagine 800, pimelic acid 200, choline hydrochloride 400, uracil 200, *i*-inositol 200, adenylic acid 40, cholic acid 200, taurocholic acid 40, cocarboxylase 4, vitamin B_6 20, vitamin B_1 100, nicotinic acid 1000, and Norit eluate factor (*9*) 40 (a preparation of which 1 microgram was sufficient for maximum growth). The recovery of pure pantothenic acid in the presence of the above mixture was 106 per cent.

As a further check on the method a number of samples of which the pantothenic acid content had been determined by the chick assay were also assayed by the bacterial method. The results are collected in Table III, from which it will be seen that the agreement was good in all cases. The data in this table also illustrate the reproducibility of results obtained by the bacterial method.

Pantothenic Acid Content of Biological Materials

The pantothenic acid content of a variety of biological materials as determined by the present method is given in Table IV, together with pertinent literature values.

Discussion

When attempts were made to use the basal medium of Snell, Strong, and Peterson (*12*) for the determination of pantothenic acid in biological materials in general, it was soon found that the bacteria were responding to substances other than pantothenic acid, or, in other words, that the medium lacked certain essential or at least stimulatory factors in addition to pantothenic acid. This inadequacy is apparent from a comparison of the curves in Figure 2. In the absence of asparagine and yeast supplement the response to pure calcium pantothenate is not linear, and the titration for 0.15 microgram is below 7 cc. Much larger amounts of the vitamin—e. g., 1 to 3 micrograms per tube—often gave titrations of 8 to 9 cc., but the response was very irregular and entirely unsuited for assay purposes.

It was therefore necessary to add a supplement to the medium which would contain an excess of the stimulating

substances but would be free from pantothenic acid. A suitable supplement was found in autolyzed yeast from which the pantothenic acid had been removed by adsorption on Norit. The complete removal of pantothenic acid from autolyzed yeast solutions proved to be difficult, and an extended series of preparations revealed that the separation could be satisfactorily accomplished only in strongly acid solution and by repeated treatment with an active Norit. Under these conditions a considerable portion of the desired stimulating substances appeared to be lost, probably both by adsorption and destruction by the acid. As a result of these difficulties the preparation of satisfactory yeast supplements, which would permit low blank and high maximum titrations, was somewhat uncertain.

The recent discovery that *l*-asparagine is one of the stimulatory substances in question (*14*) made it possible to include this chemical in the medium. With this addition and the addition of a yeast supplement prepared as above described, consistently satisfactory results have been obtained. Most of the assays reported in this paper have been carried out on a medium containing an active yeast supplement but no asparagine. Repeated tests have shown that identical results are secured whether or not asparagine is present, so long as the medium contains an adequate amount of active yeast supplement (*14*).

Throughout the present work a 3-day incubation period followed by titration of the lactic acid produced has been uniformly used to evaluate the bacterial response in preference to the quicker turbidimetric method (*6*, *10*). The reasons for this choice are twofold. First, the effect of the abovementioned stimulatory substances is greater during the first 12 to 24 hours than after the lapse of 72 to 84 hours (*14*). Although the present medium has been made as complete as feasible with respect to such stimulatory substances, the possible existence of others has not been excluded, and it is felt that the likelihood of error from this source is materially reduced by using the 3-day assay period. Secondly, when the cultures are titrated it is unnecessary to prepare a clear extract which contains all the pantothenic acid of the sample, as it is when the turbidimetric method is used. The difficulties attendant on such an extraction are especially great in the case of pantothenic acid, which appears to be largely combined in nondialyzable form in intact tissues (*8*), and which cannot be extracted with either acid or alkaline solutions because of the danger of hydrolytic destruction (*12*, *16*).

The primary standard adopted for the present assay is synthetic *d*-calcium pantothenate (*13*). Before this material became available an arbitrary standard had been employed, which consisted of a concentrate prepared from liver extract, and the pantothenic acid content of which had been determined to be 10.8 mg. per cc. by comparison with a preparation from R. J. Williams. When the older standard was assayed against the synthetic material it was found to contain 10.8 mg. per cc. This agreement further supports the accuracy of Williams' estimate of the percentage purity of his pantothenic acid preparations (*16*).

Three separate batches of synthetic *d*-calcium pantothenate have been obtained at different times, and have been found to exhibit identical potencies within the limits of accuracy of the present method. In one case the synthetic material was examined for moisture by heating over phosphorus pentoxide at 61° C. and 0.1 mm. pressure for 4 hours, whereupon the loss in weight was less than 1 per cent. There appears to be no reason why this material should not be accepted as an absolute primary standard for pantothenic acid determinations.

The reliability of the bacterial response as a measure of pantothenic acid is supported by several lines of evidence. First, the results calculated from different levels agree satisfactorily. This agreement is specifically indicated in Table I, and has also been the authors' general experience with all materials assayed. There is frequently a slight drift in these values, the direction being from higher apparent pantothenic acid content at low levels to lower results at high levels of sample per assay tube. However, the magnitude of the drift very seldom exceeds 20 per cent, and it is not observed consistently on any one type of sample.

Secondly, pantothenic acid added to various materials was satisfactorily recovered, and a series of biologically active compounds had no significant effect on the assay of pure pantothenic acid. Thirdly, there is substantial agreement between the results of chick assays and values secured by the present method (Tables III and IV). The question of possible inhibition of the growth of the organism by various materials has been previously studied (*10*), and the information obtained presumably also applies to the present assay. Furthermore, it has been shown that a high degree of structural specificity is required for pantothenic acid activity toward bacteria (*4*, *5*, *7*, *15*).

An inspection of the data in Table IV reveals a great discrepancy between the pantothenic acid content of certain materials, such as rat liver and rat muscle, as determined by the present method, and by Williams' yeast growth method (*8*), the former values being higher. However, the yeast growth results were secured on a water extract of the tissues, and presumably represent only the free pantothenic

Table III. Comparative Pantothenic Acid Assays

Sample	Pantothenic Acid Content[a] Chick assay	Microbiological Assay First	Second
	Micrograms	*Micrograms*	*Micrograms*
Yeast 1	<35	14.5	17.5
Yeast 2	>50	84	88
Sweet potato	31	26	31
Louisiana cane molasses	80	81	85
Cuban cane molasses	<10	Ca. 2	...
Cow rumen contents	90–95	88	92
Dried skim milk	46	47	44
Green split peas	19	17	12
Wheat bran 1	46	49	46
Wheat bran 2	38	39	42

[a] Per gram dry weight.

Table IV. Pantothenic Acid Content of Biological Materials

Material	Microbiological Assay	Literature Values	Method of Assay	Reference
	Micrograms per gram			
Egg yolk	72	56[a]	Chick	*1a*
Beef muscle	10	9.8	Chick	*1a*
Beef kidney	40	.....	.....	..
Dog blood	0.22	0.168[b]	Yeast growth	*8*
Human urine	2 to 6	.....	.....	..
Whole wheat	8.3	11.2	Chick	*3*
Wheat bran	27.2	25.2	Chick	*3*
Polished rice	8.8	4.2	Chick	*3*
Rice polishings	128	.....	.....	..
Yeast extract (Difco)	240	.....	.....	..
Lettuce	0.82	.....	.....	..
Rat muscle	4.41	2.72	Yeast growth	*4*
Rat muscle, autolyzed	.....	11.76	Yeast growth	*4*
Rat liver	24	2.88	Yeast growth	*4*
Rat liver, autolyzed	.....	36.0	Yeast growth	*4*
Dried buttermilk	27	.....	.....	..
Dried whey	43	56	Chick	*3*

[a] Calculated from chick assay results by multiplying "filtrate factor value" by 14 (*2*).
[b] Calculated from yeast growth results by multiplying reported values by 80 [(since Williams' "milligram unit" equals 0.08 microgram of pantothenic acid (*6*), and "yeast growth unit" is 1000 times "milligram unit" (*16*, *17*)].

acid present. After autolysis the values rise to the same order of magnitude as those found by the chick and bacterial assay methods. In view of these comparisons it seems very likely that the bacterial method measures not only the free but also the combined pantothenic acid of intact tissues.

[Since this manuscript was submitted for publication it has been found, however, that certain materials, notably yeasts and animal tissues, contain a combined form of pantothenic acid which is not available to the bacteria. Recent chick assay results on such materials have been much higher than values secured by the microbiological method as described in this paper. In order to determine the total pantothenic acid content of meats and yeast samples it is necessary first to subject them to autolysis, or to an enzyme digestion. Although the optimum conditions for the enzyme treatment are still under investigation, preliminary work indicates that 48 hours' incubation of a slightly acid (pH 5) aqueous suspension of one part of the sample plus two parts of Clarase powder (obtainable from the Takamine Laboratories, 193 Arlington Ave., Passaic, N. J.) at 37° C. gives satisfactory results. Fruits, vegetables, cereals, eggs, and dairy products do not show any significant increase in apparent pantothenic acid content when subjected to this treatment. The values for yeasts and animal tissues reported in this paper must be regarded as a measure of the free pantothenic acid present at the time the samples were assayed. The actual figures found in such cases obviously depend on the amount of autolysis which the sample has undergone before the assay is carried out.]

Although too few samples have been assayed to warrant an extended discussion of the distribution of pantothenic acid in biological materials, a few interesting points regarding the data in Tables II, III, and IV may be noted. Liver, yeast, kidney, egg yolk, dried whey, dried buttermilk, sweet potato, and the bran of grains are all good sources. Whole grains are much lower. Molasses may be very high or fairly low in pantothenic acid. The high value found for rumen contents probably indicates bacterial synthesis. In general pantothenic acid seems to be more abundant than riboflavin in foods.

The values reported for human urine are based on analysis of samples from 15 normal adult subjects of both sexes. The 24-hour urinary excretion of pantothenic acid by 10 of these subjects fell between 3 and 5 mg. with the exception of one which was 1.95 mg.

The usefulness of the assay method here reported is substantially similar to that for riboflavin (*10*). Crude suspensions of the sample are suitable for assay, so that no extraction of the pantothenic acid is necessary. Approximately 15 samples can be run by one person per day. The range of the assay is from about 0.03 to 0.12 microgram of pantothenic acid, so that very small amounts of material are needed. No unusual or expensive apparatus is required. Finally, since the identical organism and same general setup are used for the present assay as for the determination of riboflavin (*10*), it is now possible to assay samples for both vitamins with relatively little additional effort.

Summary

A rapid biological assay for pantothenic acid has been developed, which is based on the effect of this substance on acid production by *Lactobacillus casei* ϵ. The reliability of the method is supported by agreement of the assay results at different levels, recovery of added pantothenic acid, absence of any disturbing effect from other growth-promoting substances, comparison with results of chick assay on the same samples, and specificity of structure required for activity.

Acknowledgment

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Microanalysis of Gases

A Combined Toepler Pump and McLeod Gage Designed for Microanalysis with Dry Reagents

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IN CONNECTION with some work on the photolysis of gaseous acetaldehyde (*2*), a new method was desired for measurement and analysis of gas samples of the order of 5 to 500 cu. mm. (at atmospheric pressure). While no exhaustive tests have been made as to the accuracy or versatility of the apparatus, since accuracy better than about 1 per cent was not required, the method seems of sufficient interest to warrant publication.

Difficulty had been experienced in using a horizontal microburet, in the dry method of analysis of Blacet, MacDonald, and Leighton (*1*), particularly with the smaller samples. This difficulty appeared to be due to appreciable quantities of air carried into the sample with the pellet used to absorb components of the gas. In order to eliminate this source of error, as well as others of less importance, the apparatus shown in Figure 1 was constructed, combining the usual Toepler pump and an analysis system into one fairly compact unit.

Apparatus

The apparatus, which is of all-glass construction, consists essentially of a double-range McLeod gage of about 500-cc. volume with accurately calibrated volumes of 1 and 0.1 cc. marked at *E* and *F*. A chamber, *B*, of about 1.5 to 2 cc., is connected to the top of the gage by an oblique-bore 1.5-mm. capillary stopcock. This chamber, which holds the sample and reagents, is closed by a ground-glass plug, *L*, and is connected to a high-vacuum line by the stopcock, *N*.

The gage section is joined to the mercury reservoir, *D*, by a mercury-seal ground-glass joint to facilitate dismantling for cleaning. A Dewar seal is used here to reduce the height of the apparatus. As in the usual McLeod gage designed for reading higher pressures, the distance between the mercury surface in the reservoir and the calibrated capillary on the bulb should be as short as possible. The available pressure scale is determined by the distance above the calibration marks, *E*, *F*, that atmospheric pressure will force mercury in the scale capillary, *H*, about 45 cm. in this case.

The apparatus is connected at *J* to the high-vacuum line through a vacuum stopcock and to the reaction system through a mercury cutoff. (A stopcock may replace the mercury cutoff when stopcock grease is not objectionable.)

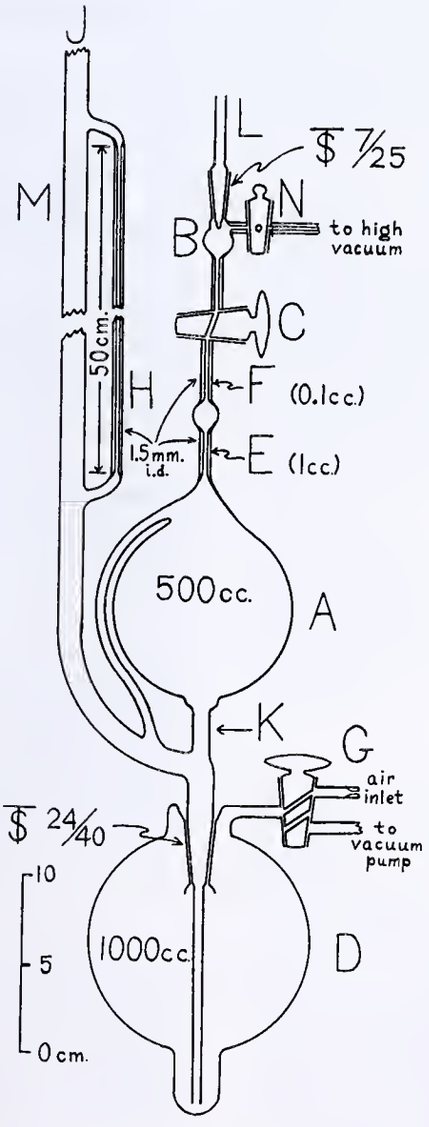

FIGURE 1. APPARATUS

Procedure

The apparatus is evacuated through *J* and *N* while separated from the reaction system by the mercury cutoff. The stopcocks to the vacuum line are then closed, the mercury cutoff to the reaction system is opened, and the gas to be measured and analyzed diffuses into *A* and *B* from the reaction system through tube *M*. Air admitted through stopcock *G* causes the mercury in the reservoir to rise, forcing the gas in *A* up into chamber *B*. Stopcock *C* is then closed and the mercury is lowered so that another portion of the gas may fill *A* and be pumped into *B*. This process may be repeated, as with the usual Toepler pump, until all of the gas to be analyzed is in the small chamber.

The sample is measured by lowering the mercury to point *K* and allowing the gas in *B* to fill the large bulb, *A*. Closing stopcock *C* causes all except about 0.4 per cent of the sample to be closed off in the large bulb, a loss which may be corrected for if desired. The mercury is then run up to one of the calibration marks, *E*, *F*. The pressure of the gas is given by the difference in height between the calibration mark used and the mercury column in *H*. Knowing the volume from the calibration and the pressure from observation, the quantity of sample is determined.

Analysis of the sample is carried out by using a dry absorbing agent such as those employed in the method of Blacet, MacDonald, and Leighton (*1*). The ground-glass plug, *L*, is removed from the chamber and a pellet of the absorbing agent—e. g., silver oxide for carbon monoxide absorption—is attached to the tip of the plug by picein or in any other convenient manner. The use of several interchangeable plugs, one for each reagent, facilitates this operation. With the plug and reagent inserted in the chamber, air is removed by opening stopcock *N* to the vacuum line. When evacuation is complete, the sample is again forced into the chamber where absorption takes place. The residue is measured in the same manner as the original sample.

In the analyses for which the apparatus has been used, some difficulty was originally experienced due to traces of moisture which were retained on the absorbing pellets (potassium hydroxide, silver oxide) during evacuation, but which appeared later when the residue was measured. Since the amount of water vapor was usually sufficient to saturate the small volume to which the gas is compressed during measurement, it was found convenient to permit this saturation to take place and then subtract the water vapor pressure from the observed pressure rather than use an extra pellet for drying the gas.

While the present apparatus has been employed only in the analysis

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Table I. Analyses for Carbon Monoxide

(Rollefson and Grahame, 3, found 49 ± 0.3%)

Sample	Residue	CO
Cu. mm.	*Cu. mm.*	%
112.2	56.6	49.6
327	162.4	50.3
313	159.0	49.2
69.8	35.0	49.9
190.8	95.5	49.8
479	245	48.8

of carbon monoxide, the method should be applicable to any analysis in which liquid reagents may be avoided.

A few typical analyses for carbon monoxide are shown in Table I. No attempt has been made to improve the results obtained, but it is believed that increased time of preliminary evacuation and removal of water vapor would lead to improved performance.

There are a number of possible designs that may be applied to the reaction chamber to increase the versatility of the apparatus. For example, ground joint *L* could be replaced by a spherical ground joint, its female section being connected to stopcock *C*. The male section would have any desired number of indentations on its ground surface, within which could be attached pellets of the same or various reagents. In the first case, this would enable the reagent to be renewed merely by exposing a different indentation to the reaction chamber. In the second case, should the gas contain a number of components, reagents for removing only certain components could be exposed to the reaction chamber as desired. It is also possible that an explosion, a combustion, or an oxidation reaction chamber may be designed for the apparatus.

Acknowledgment

The authors wish to express appreciation for the advice and encouragement of O. K. Rice in the design and construction of this apparatus.

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Separation of Calcium Nitrate from Strontium Nitrate

By Monobutyl Ether of Ethylene Glycol

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IN SEPARATING and identifying barium, calcium, and strontium after these elements have been precipitated as carbonates, use is made of the differential solubilities of the salts of the alkaline earths or the selective solubilities of organic reagents on the anhydrous chlorides or nitrates of these metals. While the different procedures have given fairly satisfactory separation of the three alkaline earths, they have not offered a clear-cut and positive separation. The uncertainty has been especially marked with students in analytical chemistry and with the analyst who does not have occasion to make frequent separations of the alkaline earth metals.

Although the differential solubilities of the alkaline earth salts cannot be changed appreciably by varying the concentration of the separating reagents, the selective solubility of organic reagents on the anhydrous chlorides or nitrates should afford a positive separation if a reagent could be found which had a high solvent action on, say, the nitrate of calcium and a negligible, or exceedingly low, solvent action on the nitrate of strontium.

Anhydrous alcohol-ether mixtures (*4*), isopropyl alcohol (*2*), amyl alcohol (*3*), acetone (*5*), butyl alcohol (*6*), etc., have afforded a fair separation of the anhydrous chlorides or nitrates of the alkaline earths. However, the ether-alcohol mixture is rather dangerous on account of its easy inflammability. With these organic solvents the anhydrous solvent must be prepared and protected from the moisture of the atmosphere and should be used without moisture contact in order to keep the selective solvent action at a maximum-minimum ratio. Even then, these reagents do not give a complete separation of the anhydrous chlorides or nitrates of the alkaline earths.

An investigation of other solvents was undertaken, and the monobutyl ether of ethylene glycol (butyl Cellosolve) was found to have the desired properties. This reagent gives a complete separation of the anhydrous nitrates of strontium and calcium and, their hydrated nitrates are rendered anhydrous at, or below, the boiling point of the solvent (170.6° C.). Furthermore, the anhydrous solvent is obtained by heating it to the boiling point when dehydrating the calcium and strontium nitrates—that is, the calcium and strontium nitrates and the solvent are rendered anhydrous in one operation. Because of this double action, it is not necessary to dehydrate the nitrates by heating them at uncertain temperatures, and then attempt to dissolve the more soluble nitrate by triturating the baked nitrates in the solvent which is absorbing moisture from the air as well as from the vessel in which the salts were dehydrated.

The Reagent

Commercial butyl Cellosolve having a boiling range from 163° to 174° C. was used. About 96 per cent distills below 172°. It is water-white, somewhat hygroscopic, soluble in water in all proportions, does not have an unpleasant odor, is not easily ignited, but will burn when vaporized into a flame. The pH of the material used was 5.8. The inflammability does not appear to be dangerous or explosive, when the solvent is used as directed. The vaporized Cellosolve should be ignited when it is boiled off.

Semimicroprocedure for Analysis of Alkaline Earth Group

The alkaline earth group will be contained in the filtrate from the previous group precipitation. This filtrate will be ammoniacal and will contain ammonium sulfide.

Add 1 drop of neutral red indicator (pH 6.8 to 8.0) and boil the solution until it turns from yellow to red and becomes clear. [On boiling, the decomposition of the ammonium sulfide results in a deposit of sulfur (white), but when the solution becomes distinctly red, the white deposit disappears, and the solution becomes clear.] Make the volume of the filtrate to about 2 ml. by

PRECIPITATE: $BaCO_3$, $CaCO_3$, and $SrCO_3$. Treat the precipitate with a few drops of dilute acetic acid and wash the filter with 1 ml. of water. To the filtrate add 2 drops of a saturated solution of ammonium acetate and 2 drops of 1 *N* potassium chromate reagent. Shake the tube; if a precipitate is formed filter and wash the precipitate with water. [The precipitate should be washed with 3 to 5 ml. of water. Collect about 0.5 ml. of this water in the filtrate and discard the remainder.]

PRECIPITATE: $BaCrO_4$. Dissolve in 1 ml. of 0.1 *N* HCl. Add 1 drop of formalin solution, place the tube in the hot water bath, add several drops of a saturated solution of calcium sulfate. A white precipitate confirms the presence of barium. [The formaldehyde reduces the CrO_4^{--} (yellow) to Cr^{+++} (green) and enables one better to observe the formation of the white ($BaSO_4$) precipitate.]

FILTRATE: calcium and strontium ions. Add 1 drop of neutral red indicator and then concentrated ammonium hydroxide until the solution is yellow. To this yellow solution add 5 drops of 1 *N* sodium carbonate reagent. Place the tube in the hot water bath until the precipitate has settled. Filter and wash the precipitate with water to which a drop of sodium carbonate has been added.

PRECIPITATE: $CaCO_3$ and $SrCO_3$. Dissolve in a drop or two of dilute nitric acid and wash the filter with about 1 ml. of water. Evaporate the filtrate to incipient dryness by use of the burner flame. [Do not evaporate to complete dryness—i. e., avoid overheating the calcium and strontium nitrates.] To this practically dry residue add 10 drops of butyl Cellosolve, and again evaporate to incipient dryness. To the residue add 1 ml. of butyl Cellosolve and bring to boiling. Filter the hot solution (caution) through a dry filter (*) medium, glass or cotton (*1*). Add to the test tube another milliliter of butyl Cellosolve; heat to boiling and filter through the same filter which was just used.

FILTRATE: $Ca(NO_3)_2$. Add 1 drop of oxalic acid reagent. A white precipitate shows the presence of calcium.

RESIDUE: $Sr(NO_3)_2$. To the residue add 1 ml. of water and filter through (*) above. To the filtrate add 2 drops of ammonium sulfate reagent. Place the tube in the hot water bath and allow to stand for a few minutes. A white precipitate confirms the presence of strontium.

adding or boiling off water. Add 1 drop of concentrated ammonium hydroxide and 5 drops of 2 *N* ammonium carbonate reagent. Place the tube in the hot water bath until the precipitate has settled (*1*). To the clear solution above the precipitate add 1 drop of ammonium carbonate. If a precipitate appears in the clear solution, add ammonium carbonate until precipitation is complete. Filter and wash the precipitate with water to which a drop of ammonium carbonate has been added. (See above.)

Solubilities

Table I gives comparative solubility (grams per ml.) of a few common salts of the alkaline earths in water and of the anhydrous nitrates in butyl Cellosolve. The solubility of the anhydrous nitrates of barium, calcium, and strontium is based on the following procedure.

A drop of a 0.1 *M* solution of barium and strontium nitrates was boiled in about 1 ml. of the reagent until the salt and the reagent were rendered anhydrous. (This is accomplished before the reagent is entirely evaporated.) To the anhydrous salt 5 ml. of the reagent were added and brought to boiling. The hot reagent was filtered through a dry filter medium (glass wool), 1 ml. of the filtered reagent was evaporated to dryness, the residue was dissolved in 1 ml. of water, and to this solution a drop of ammonium carbonate for strontium and a drop of ammonium sulfate for barium were added. No precipitate was noticeable in either test after allowing to stand for 5 minutes. Attempts to determine the solubility by evaporating 10 ml. of the anhydrous reagent, which had been saturated with anhydrous barium and strontium nitrates, and weighing the residue gave very low but variable and uncertain results. This appeared to be due to a slight decomposition of the reagent on evaporation which resulted in some carbon deposits.

TABLE I. SOLUBILITIES OF ALKALINE EARTH SALTS

	Barium	Calcium	Strontium
Carbonate	2.2×10^{-5}	1.5×10^{-5}	1.1×10^{-5}
Chromate	3.4×10^{-6}	1.6×10^{-1}	1.2×10^{-3}
Oxalate	9.3×10^{-5}	6.7×10^{-6}	5.1×10^{-5}
Sulfate	2.3×10^{-6}	2.4×10^{-3}	1.1×10^{-4}
Nitrate (in butyl Cellosolve)	Not more than 2.3×10^{-6}	2.43×10^{-1}	Not more than 1.1×10^{-5}

The solubility of the anhydrous calcium nitrate was determined by saturating the anhydrous reagent with the anhydrous calcium salt, evaporating 1 ml. of the clear, saturated reagent to dryness, and weighing the residue.

Summary

Calcium nitrate is completely and easily separated from strontium nitrate by the use of the monobutyl ether of ethylene glycol. The hydrated calcium and strontium nitrates and the reagent are rendered anhydrous by boiling the nitrates in the reagent (b. p. 170.6° C.).

The solubility of anhydrous calcium nitrate in the reagent is 2.43×10^{-1} gram per ml., while the solubility of the anhydrous barium and strontium nitrates in the anhydrous reagent is not more than that of strontium carbonate and barium sulfate in water.

An analysis of 25 unknowns of the alkaline and alkaline earth groups, using 1 drop of the unknown solution of 0.1 *M* concentration, gave results 100 per cent correct, and without uncertainty as to the presence or absence of the alkaline earth metals.

Acknowledgment

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(4) Fresenius, *Z. anal. Chem.*, 32, 191 (1893).
(5) Sneed, M. C., private communication.
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Report on Recommended Specifications for Microchemical Apparatus

Carbon-Hydrogen and Dumas Nitrogen

G. L. ROYER, CHAIRMAN, H. K. ALBER, L. T. HALLETT, W. F. SPIKES, AND J. A. KUCK, SECRETARY

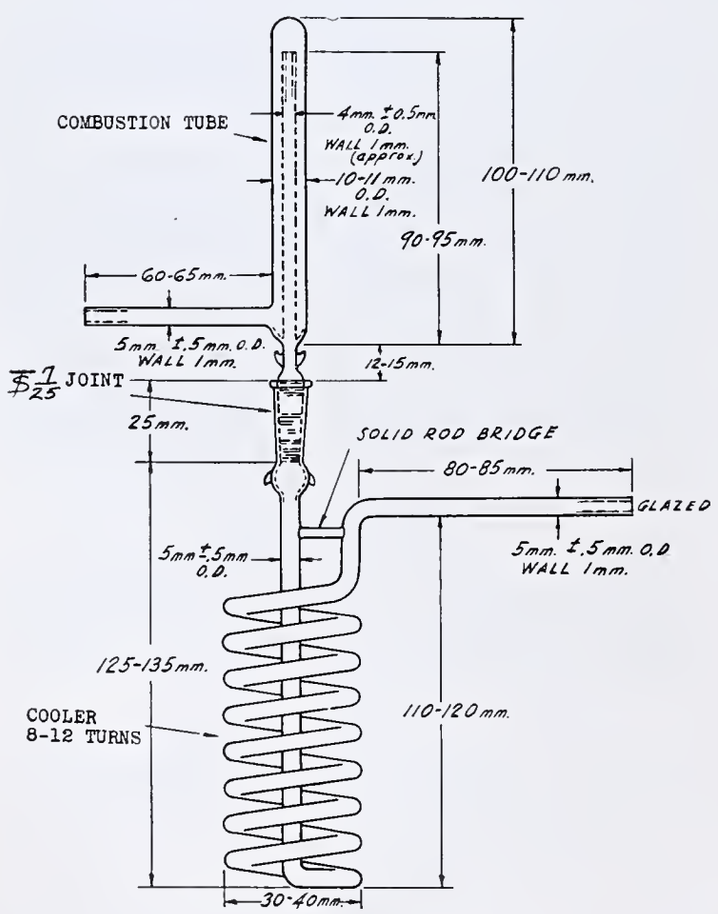
AT THE Rochester meeting of the AMERICAN CHEMICAL SOCIETY in September, 1937, a committee for the standardization of microchemical apparatus was appointed by W. R. Kirner, who was then chairman of the Division of Micro Chemistry. The purpose of this committee was to investigate the wide variation in dimensions of various common pieces of apparatus which are employed in organic microanalysis and to suggest to the division recommended specifications of such apparatus in so far as it seems practical to do so.

Microchemistry has now progressed to such a point that standardization of apparatus for the common analytical procedures is both desirable and possible. The use of recommended apparatus should contribute to the progress of microchemistry. The microanalyst, particularly a beginner in the field, relies to a great degree on correctly dimensioned apparatus for the success of his results. It is a well-established fact that failures in microanalytical procedure have occurred because of insufficient consideration for even small changes in design and dimensions. Individual pieces of apparatus must be not only properly constructed in themselves but capable of being connected to each other to form a satisfactory assembly.

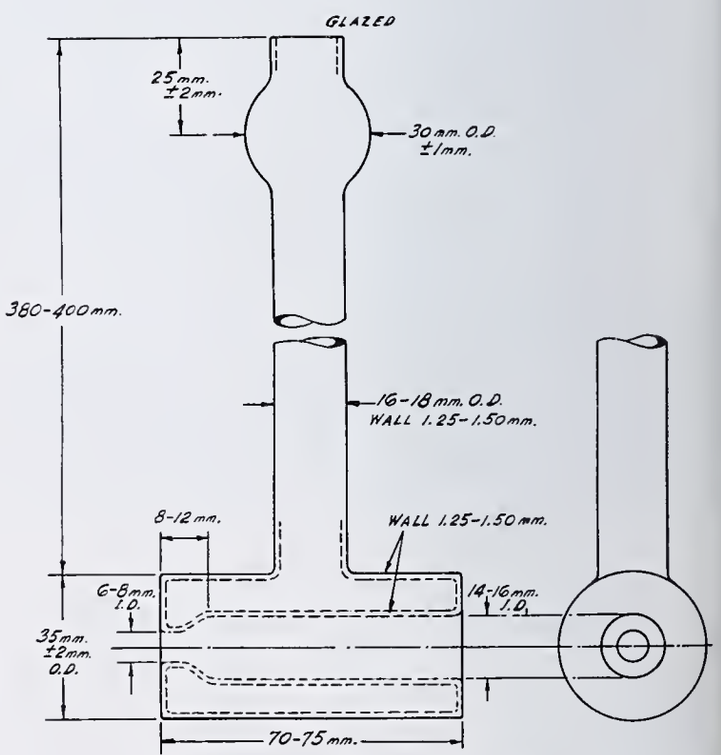
Apparatus made according to recommended specifications will also be desirable from the manufacturer's point of view. It will make possible simplification of the types of units which are kept in stock, with a resulting economy for the consumer. It will assist the manufacturer in constructing correctly designed equipment for this new and highly specialized field. Until recently, much of the microchemical apparatus in use in America was imported from foreign countries where microchemistry had an earlier start. The necessity for importation was partly due to a lack of information on the part of the American manufacturers. It is hoped that these recommended specifications will enable American microanalysts to obtain domestically manufactured equipment and replacement items suitable for use, which at present cannot be imported.

In developing these recommended specifications, consideration was given to the type of unit now in general use, cost, simplicity of construction, durability, and efficiency in service. It was not the idea of the committee to set up apparatus as "standard apparatus" which could not be changed or varied in carrying out a determination, but to set up recommended specifications for each unit of assembly. It is hoped that improvements will continue to be made in the procedures and apparatus and that new ideas, after publication of separate papers, may again be considered by the committee and at some future time be presented as revised specifications.

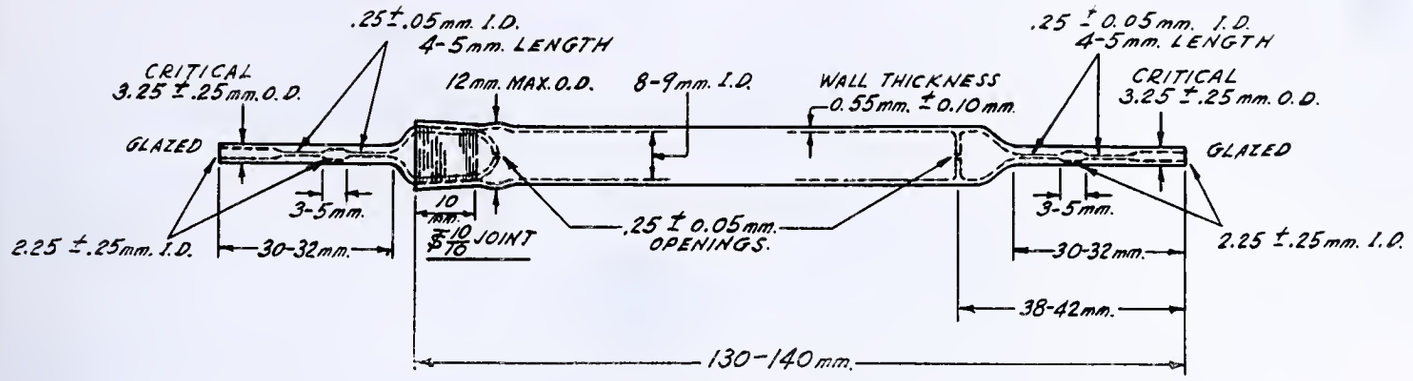
Preliminary mimeographed reports along with drawings were presented to the members of the Division of Micro



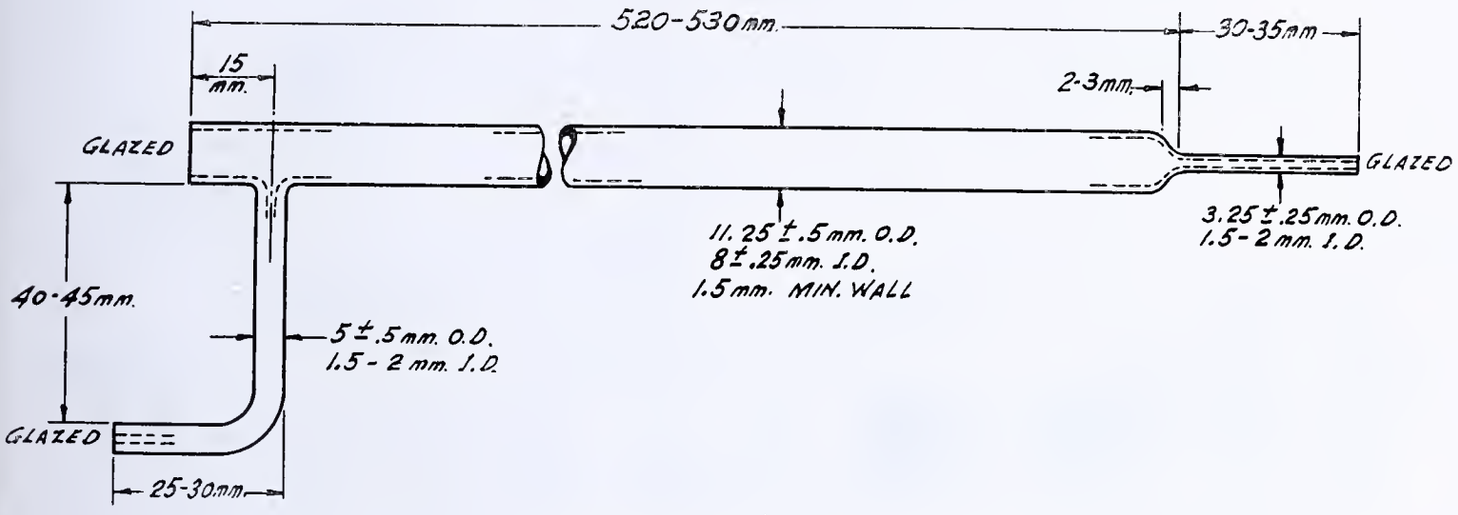
PREHEATER



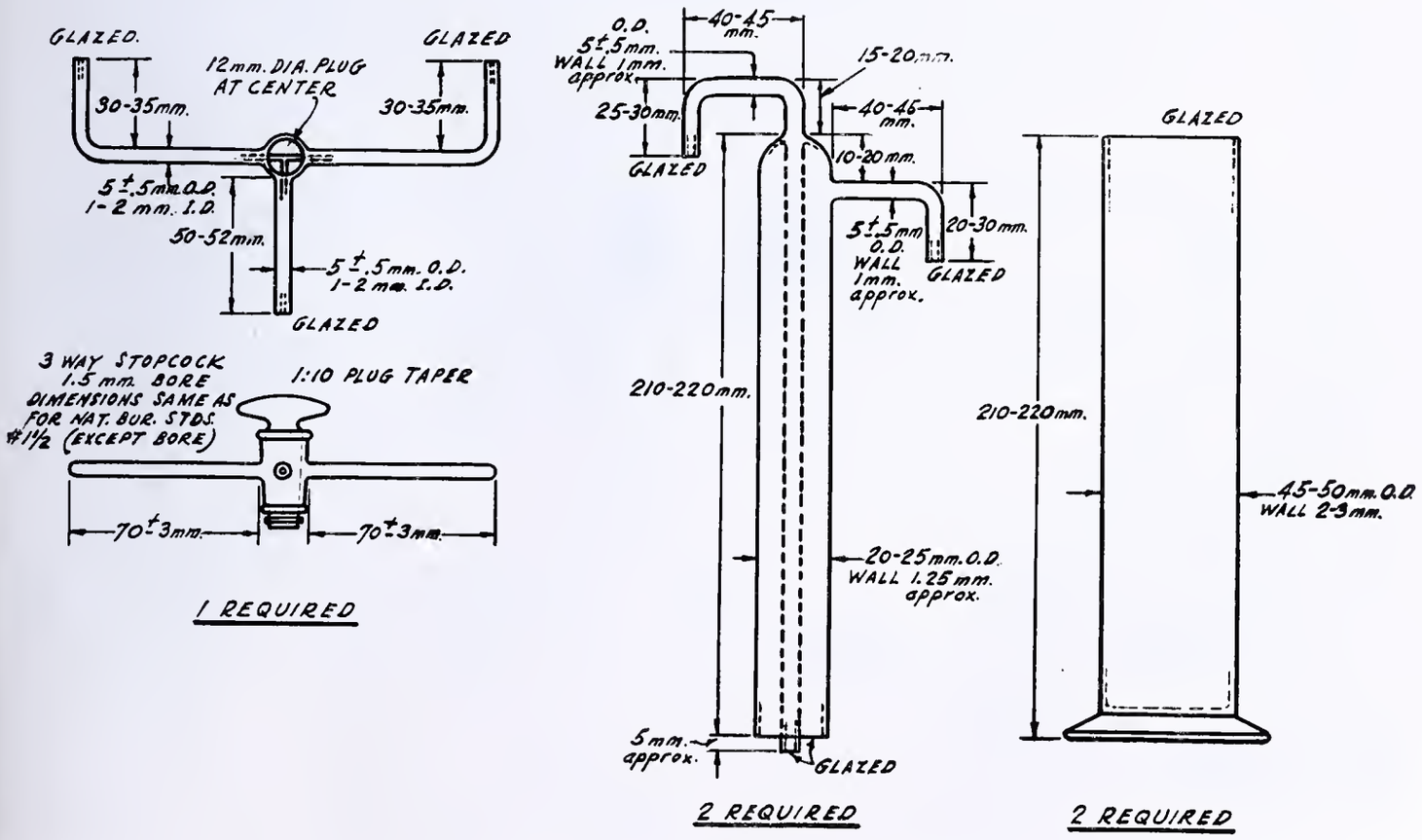
CONSTANT-TEMPERATURE CHAMBER



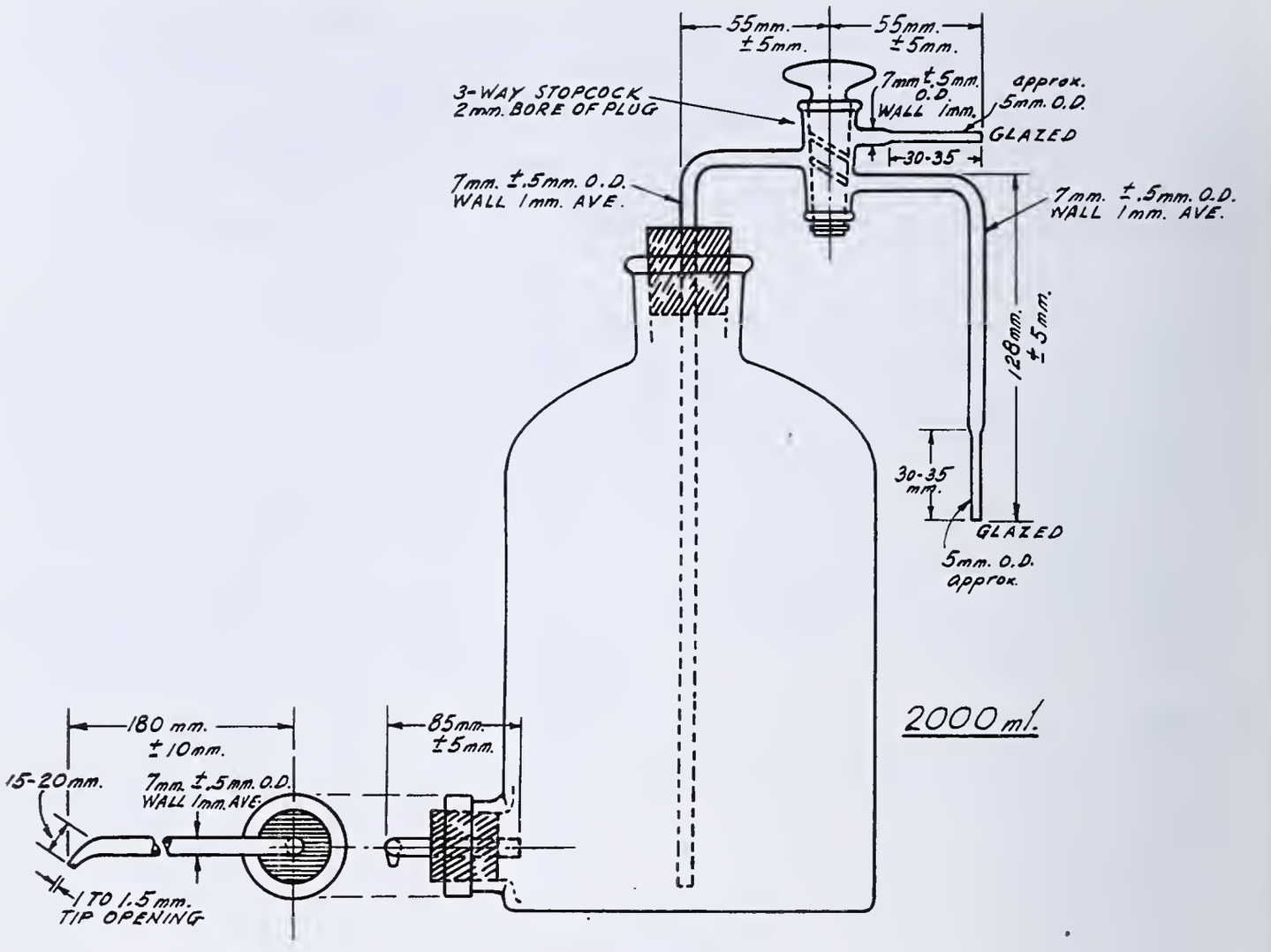
ABSORPTION TUBE
Total weight of empty tube 6 to 8 grams



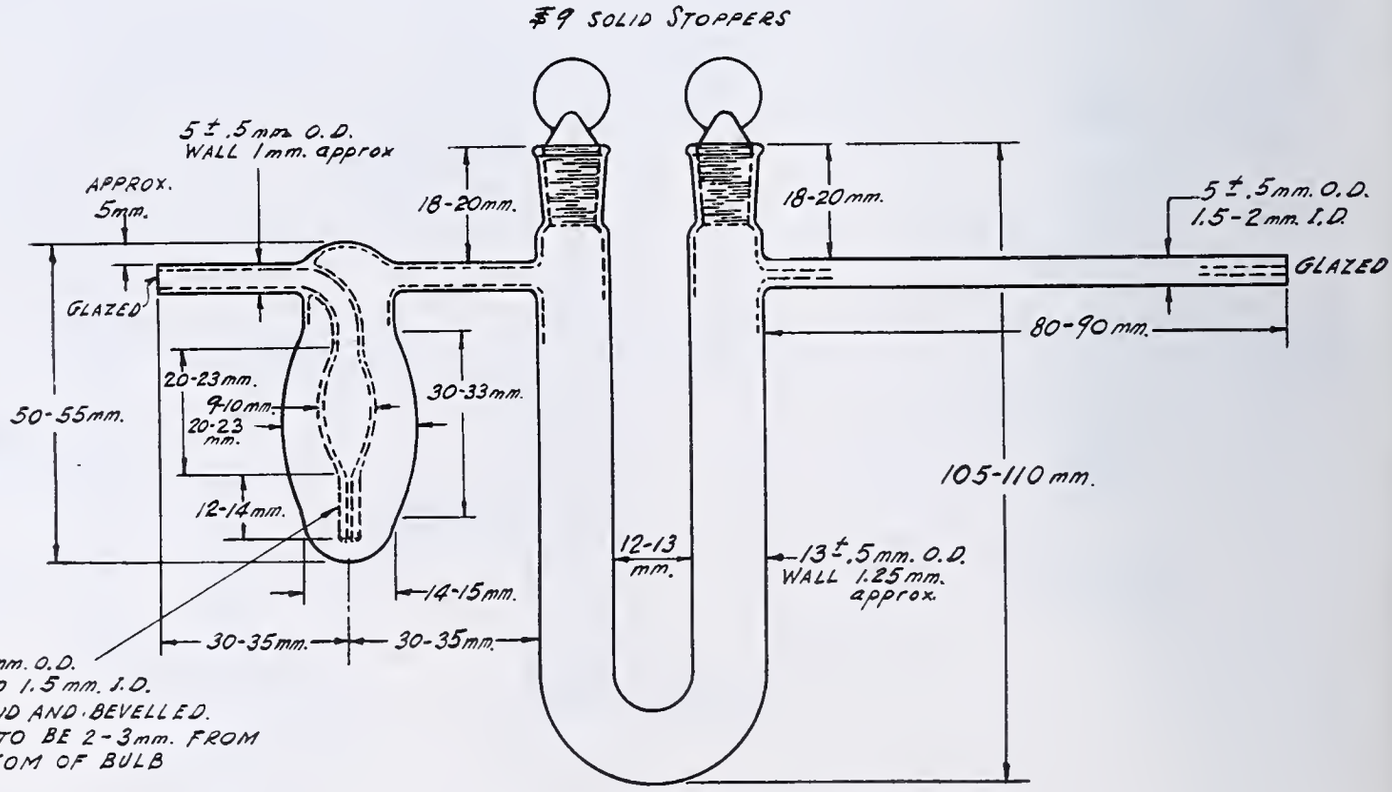
COMBUSTION TUBE WITH SIDE ARM



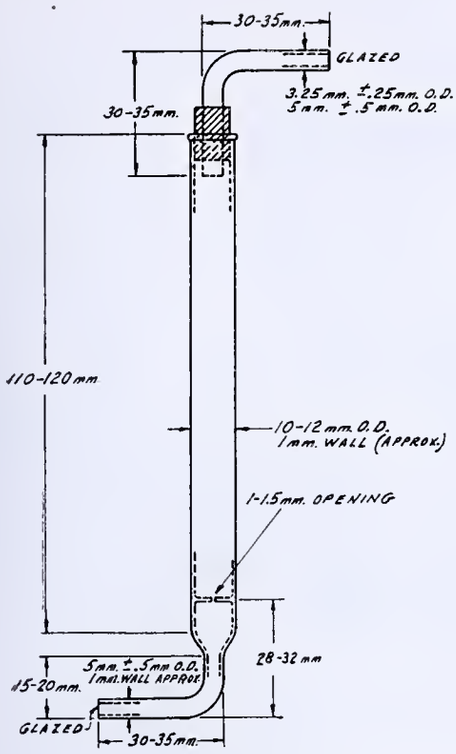
GLASS PARTS FOR PRESSURE REGULATOR



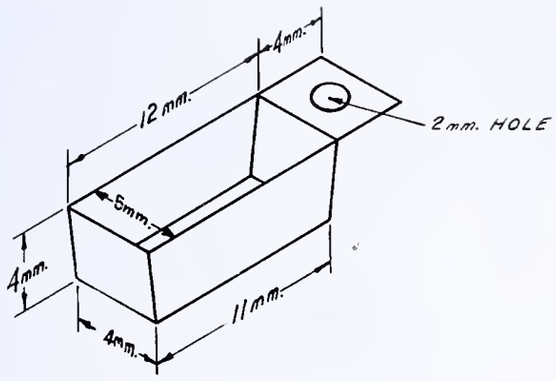
MARIOTTE BOTTLE



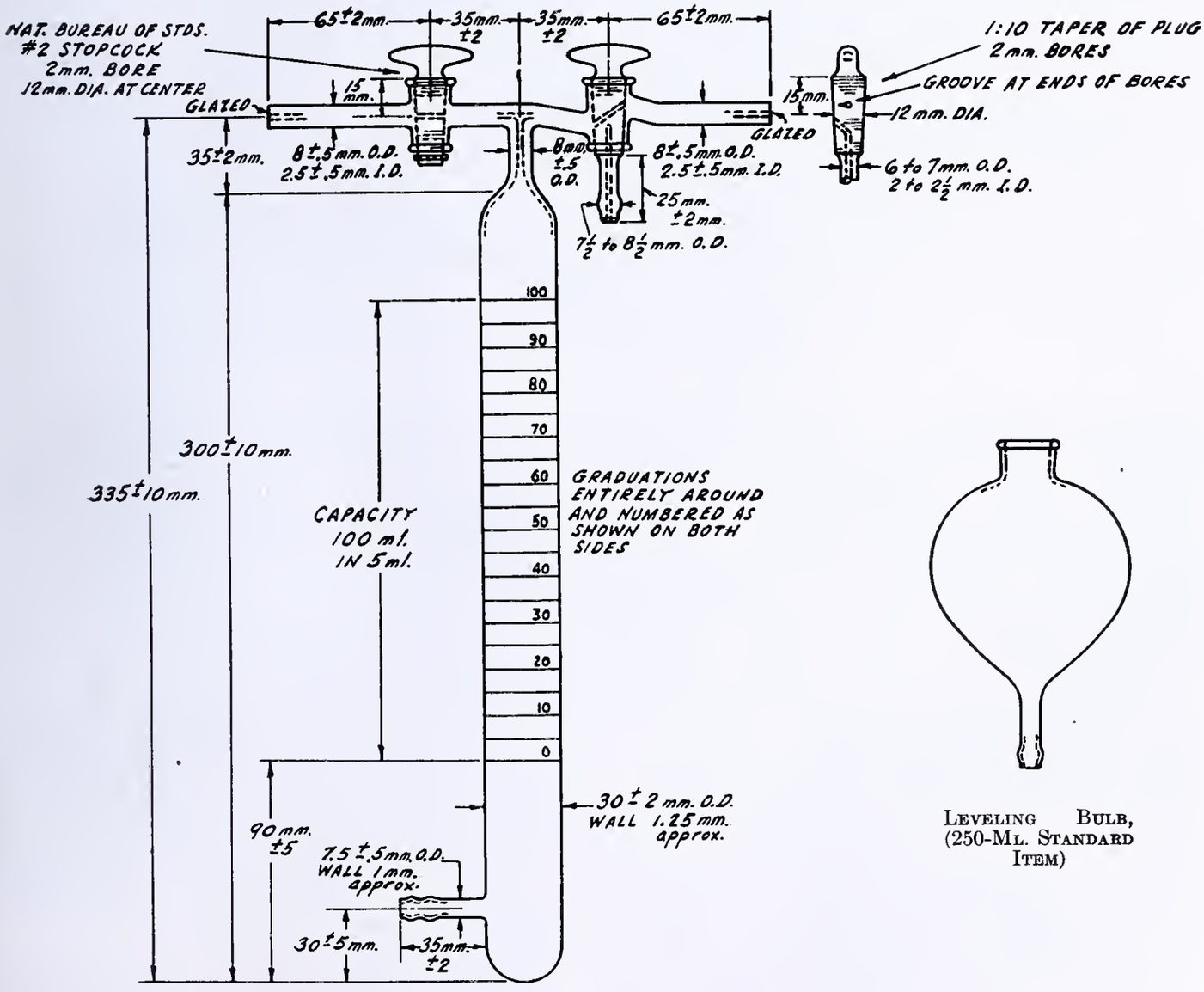
BUBBLE COUNTER—U-TUBE



GUARD TUBE

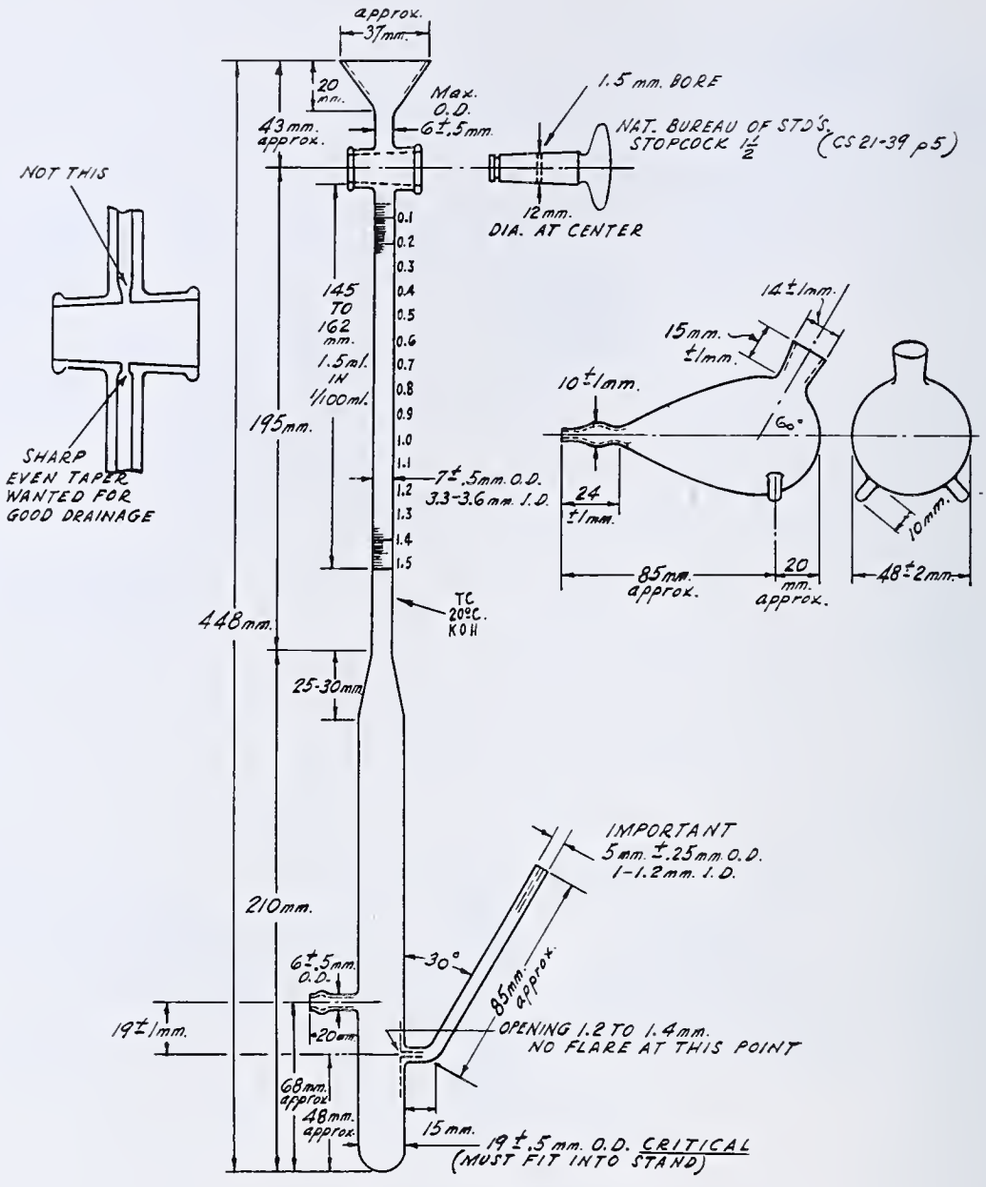


PLATINUM BOAT
Weight, 0.7 to 1 gram
3.5% rhodium alloy



GASOMETER

LEVELING BULB, (250-ML. STANDARD ITEM)



PRECISION NITROMETER AND LEVELING BULB

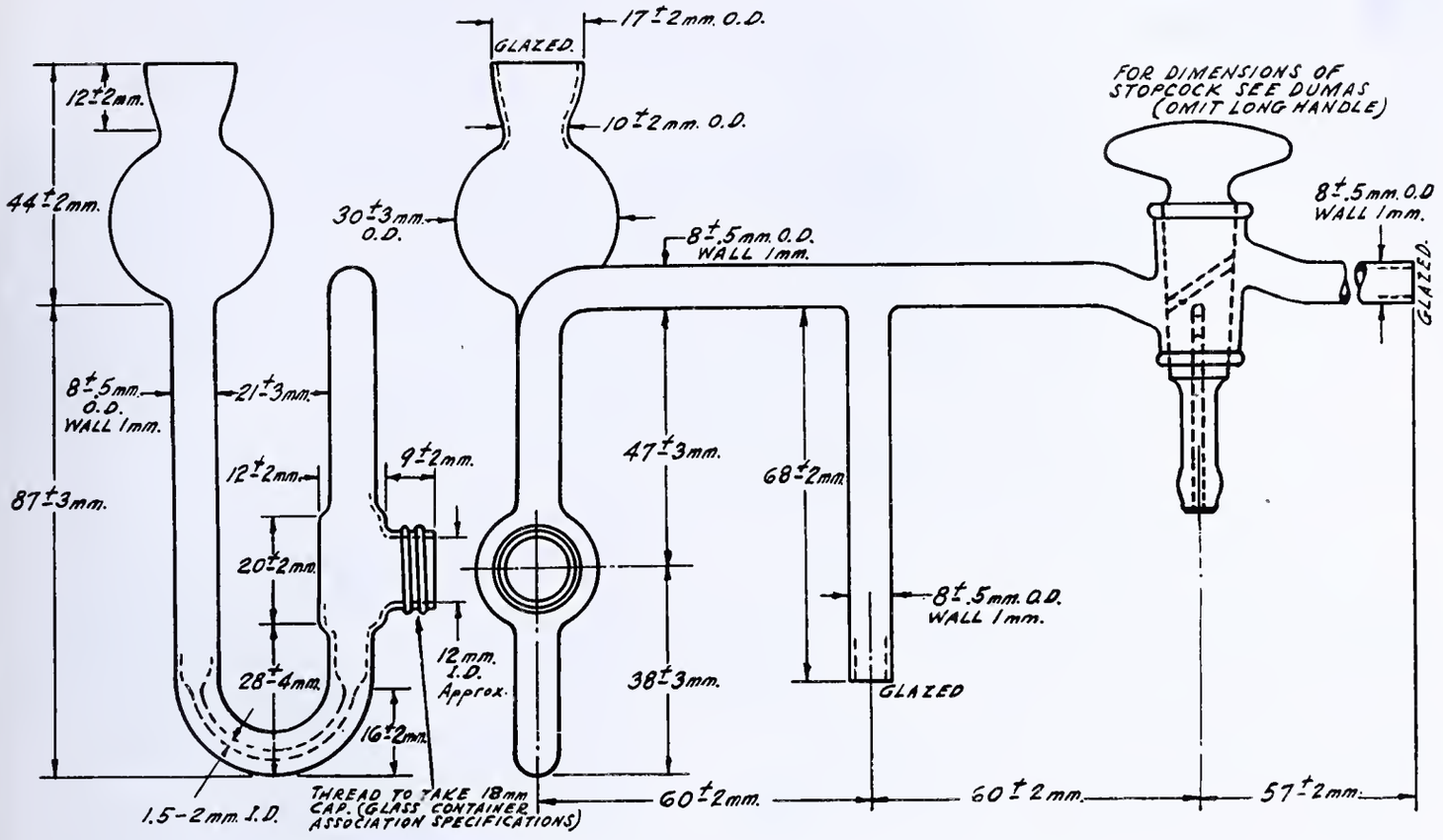
Chemistry for their comment. Criticisms of these reports sent in by various manufacturers and users of apparatus were reviewed. A revised preliminary report was forwarded to a special committee of the Scientific Apparatus Makers of America, appointed by John M. Roberts, president, which consisted of J. J. Moran, chairman, A. A. Kelm, L. D. Wilson, W. B. Warren, and J. E. Patterson. The specifications reported here have been approved by this committee and thus automatically receive the approval of the Scientific Apparatus Makers. With the acceptance of these recommended specifications by this organization and with the approval of the Division of Analytical and Micro Chemistry, the Committee on Standard Apparatus of the AMERICAN CHEMICAL SOCIETY advocated their publication.

Since the main idea of this report was to present recommended specifications for apparatus, it was thought that all information should be included on the drawings. In this way all the important information is assembled, so that the drawings alone are sufficient for any manufacturer's use. It is apparent upon consideration of the function of these individual pieces of apparatus that certain dimensions are theoretically and practically critical, while others may be varied within wide limits. However, dimensions which may not be in themselves critical can be accurately controlled in the manufacturing process without any inconvenience and therefore all dimensions are specified with limits, which were agreed upon by both manufacturers and microchemists.

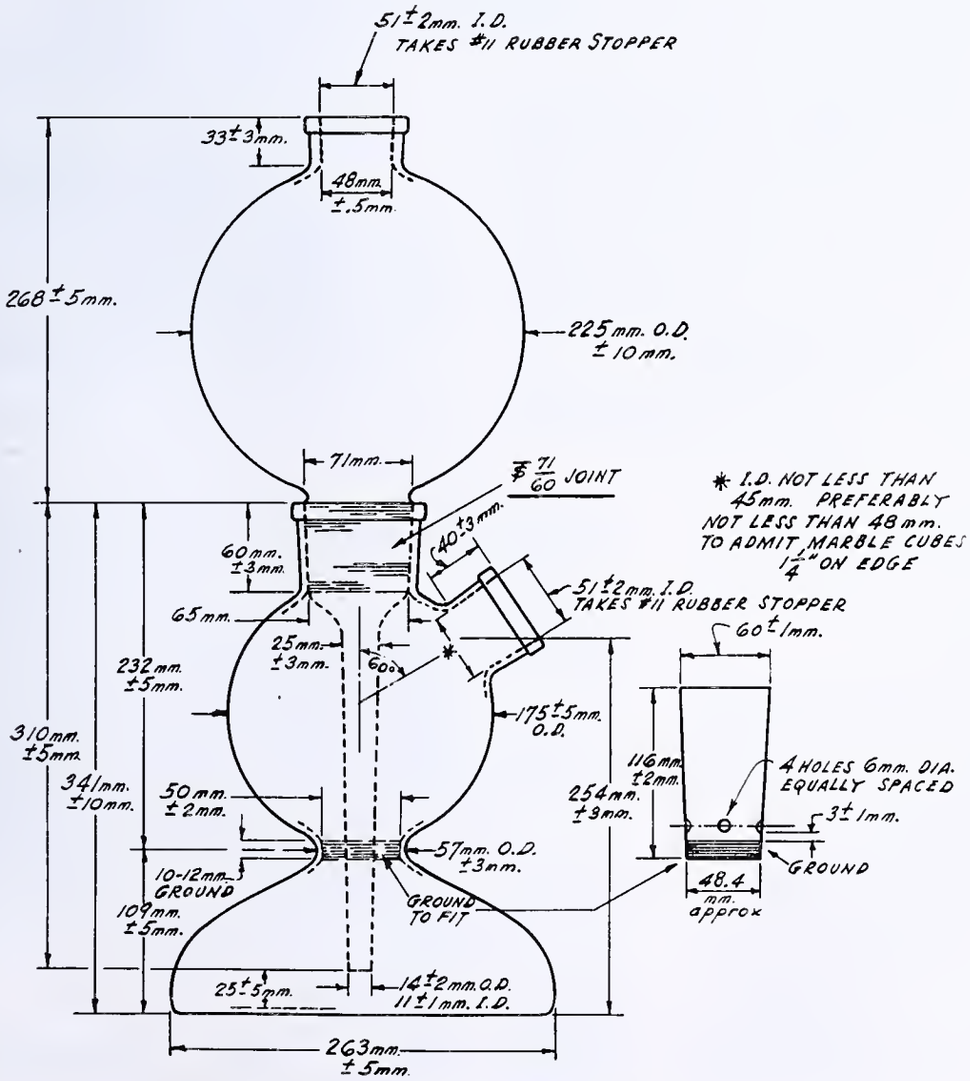
It is not thought desirable, nor is it possible, to list in this publication the reasons for the choice of the recommended specifications; nor is it possible to give credit to the originator of every piece of apparatus which has been considered. In the bibliography which follows at the end of this report, the committee has tried to give credit to its chief sources of information. However, these references cannot be considered as primary sources in themselves, since they also contain earlier references in the scientific literature to specific details of design. In many cases a piece of apparatus described in this report has been modified, in the hands of successive workers, over a period of time, so that the name of the actual originator has been lost. For this reason each piece of apparatus in this report has been given a descriptive name without reference to any one individual's design.

The units for the carbon and hydrogen determination and the nitrogen determined by the Dumas method which are considered in this report are those used for the Pregl technique but can, in most cases, also be applied to the various modifications suggested by other authors.

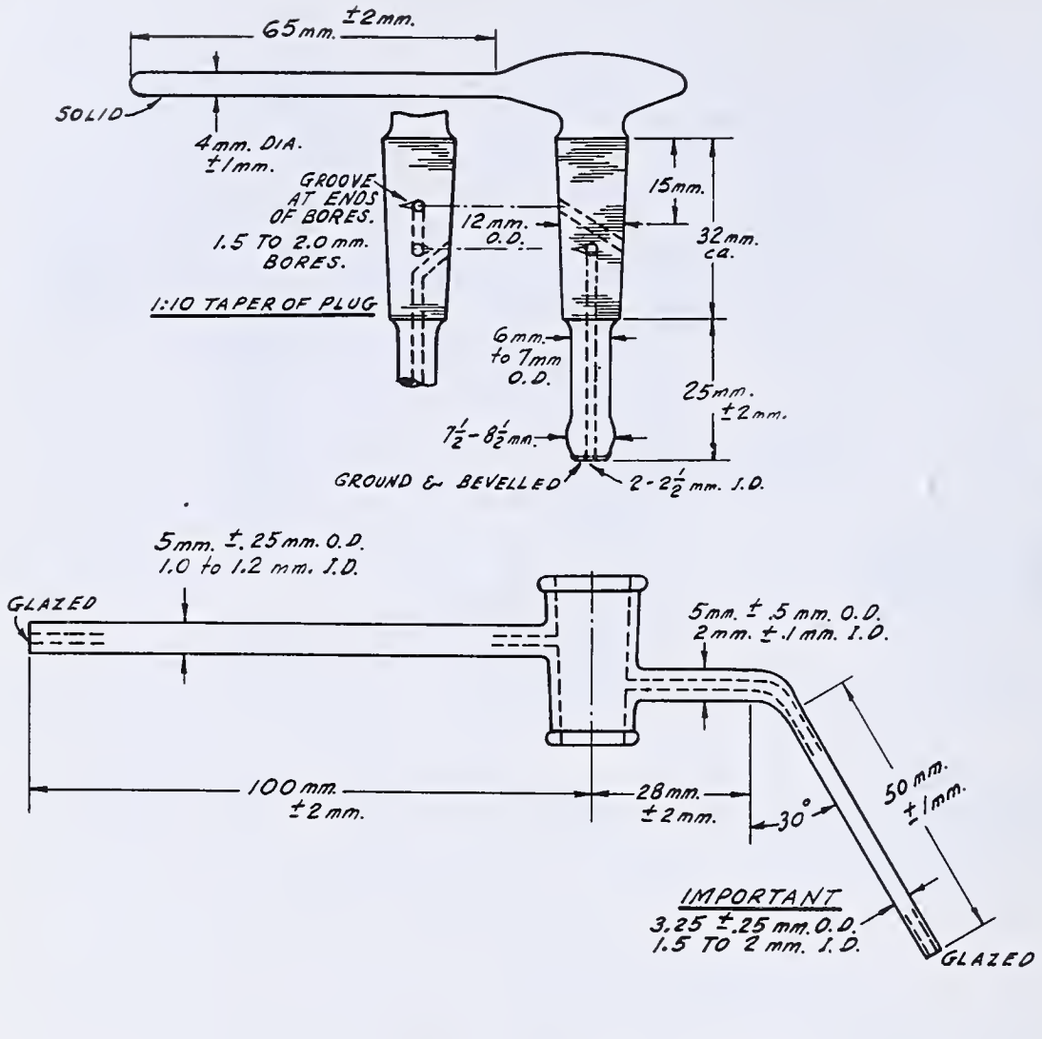
The drawings which accompany this report are not reproduced here in full size. Copies of the full-size drawings may be obtained at a nominal cost by writing to the office of



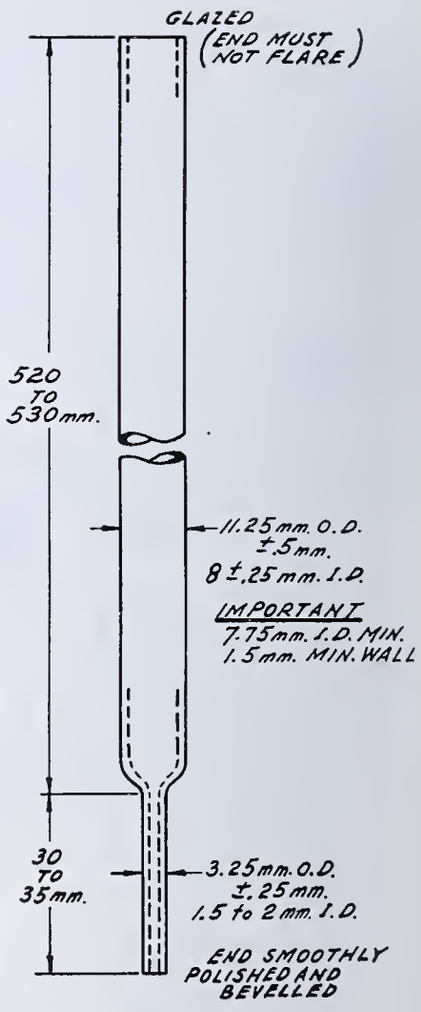
Mercury Valve for Carbon Dioxide Generator for Use with Dry Ice



Standard Kipp Generator, 2000-Ml.



Stopcock for Dumas


GLAZED (END MUST NOT FLARE)
520 TO 530mm.
11.25mm. O.D. ±.5mm.
8 ±.25mm. I.D.
IMPORTANT 7.75mm. I.D. MIN. 1.5mm. MIN. WALL
30 TO 35mm.
3.25mm. O.D. ±.25mm. 1.5 to 2 mm. I.D.
END SMOOTHLY POLISHED AND BEVELLED

Combustion Tube with Tip

the Scientific Apparatus Makers of America, 20 North Wacker Drive, Chicago, Ill.

Part I. Carbon-Hydrogen

The following units were considered by the committee as requiring specifications.

The glass parts for pressure regulator
The preheater
The bubble counter–U-tube
The combustion tube with side arm
The constant-temperature chamber
The absorption tube
The guard tube
The Mariotte bottle
The platinum combustion boat

Part II. Dumas Nitrogen

In continuing the work of the standardization committee, the following recommended specifications are presented for the apparatus used in the Dumas nitrogen procedure:

Standard Kipp generator
Mercury valve for carbon dioxide generator for use with dry ice
Gasometer and leveling bulb
Combustion tube with tip and stopcock for Dumas
Precision nitrometer and leveling bulb

Acknowledgment

In addition to the references given below the committee makes grateful acknowledgment of aid received from the members of the Division of Analytical and Micro Chemistry, and of personal suggestions and discussion with members of the Pregl group of the Metropolitan Microchemical Society of New York, particularly to Joseph B. Niederl, associate professor of chemistry, New York University. The committee also acknowledges the financial assistance of the manufacturers who contributed toward the expenses connected with the distribution of the preliminary reports and the support of the National Youth Administration under whose auspices the preliminary drawings were made.

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A Microchamber for Low Temperatures

ZABOJ HARVALIK, St. Ambrose College, Davenport, Iowa

IN ANALYTICAL and preparative chemical work it is often necessary to use fractional crystallization by low temperatures and to determine the physical constants of the crystals frozen out. The microchamber described (Figures 1 and 2) makes it possible to observe through the microscope any object under the influence of low temperatures in the range of +80° to −180° C. It can be attached to every type of microscope and can be used for any chemical and biological work where low temperatures are required (*1*, *2*, *3*, *6*, *7*, *9*, *13*, *14*, *18*).

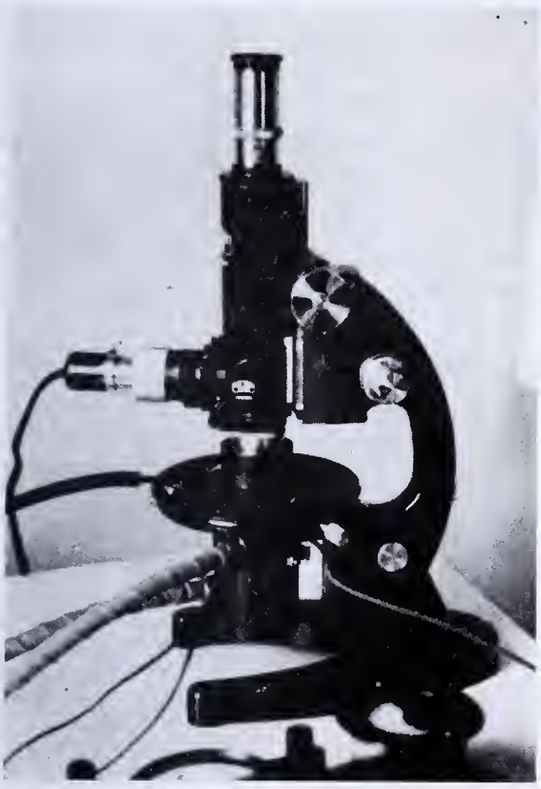

FIGURE 1. MICROCHAMBER ATTACHED TO MICROSCOPE

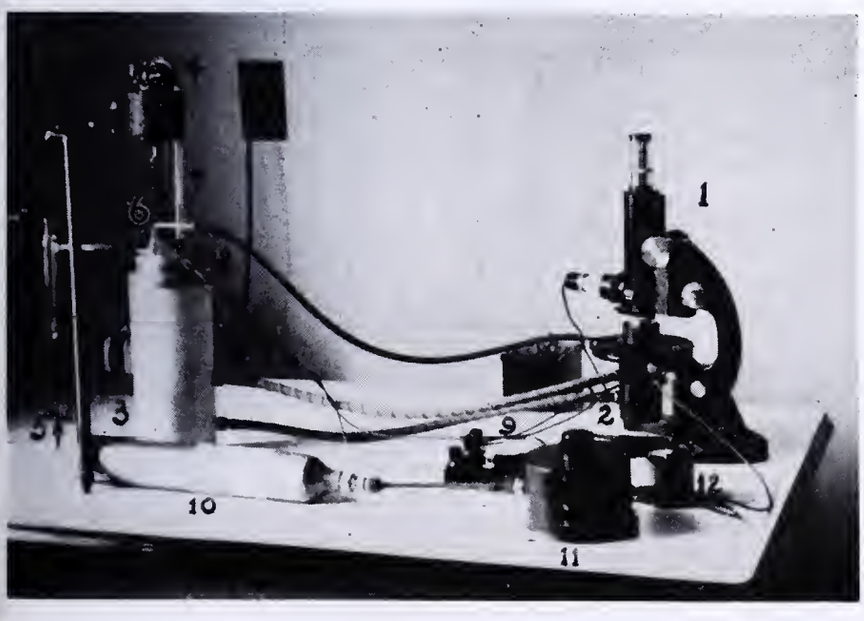

FIGURE 2. MICROCHAMBER IN OPERATION

1. Microscope
2. Microchamber
3. Storage vessel
4. Electric motor, variable speed
5. Bubble counter
6. Condenser
7. Thermometer
8. Thermocouple
9. Microvoltmeter
10. High-pressure tank
11. Pressure regulator
12. Manometer

The microchamber shown in Figure 3 is used on a microscope with illumination from above. The chamber shown in Figure 4 is for microscopes with illumination from below. To attach the microchamber to this type of microscope, the condenser and the mirror must be taken off. The illumination of the sample in the chamber is provided by a Lucite rod of given shape (*15*).

Description of Microchamber

The microchamber is based upon the fact that objects in rooms surrounded by walls at constant temperature get the same temperature as the walls. A constant temperature is maintained by circulating liquids around them.

A copper rod shaped as in Figure 3, 10, is surrounded by a metal tube, 20, which has two openings, 21, 22, in its side walls for the inflow and outflow, respectively, of a cooling fluid. This cooling fluid comes from a storage vessel (Figure 5) through the lower metal hose and flows back through the upper one. A spiral, 23, enables better circulation of the cooling fluid around the copper rod.

The temperature in the chamber is measured by a thermocouple which is soldered directly in the copper rod, 13. The sample is placed in a thin-walled vessel, 11, covered with a cover glass, inserted in the chamber, and covered with a copper ring, 101. The copper ring insulates the sample against thermal influences from above and maintains a constant temperature in the chamber (*11*).

The chamber and its accessories are protected by a metal or Bakelite covering to avoid thermal losses. This covering, 31, is attached to a metal tube, 30, by cork insulation, 24. Tube 30 itself serves as a means of attaching the whole chamber to the microscope stage by a bayonette lock to a plate, 32, which is screwed, 33, on the microscope stage, 40.

The space between the sample and the objective should be filled with slowly flowing dry gas—e. g., air, carbon dioxide, nitrogen, or argon—to avoid precipitation of frost from the air. The gas flows through tube 51 into the open space formed by tube 50, and the overflowing dry gas prevents moist air from reaching the chamber or the cover glass over the sample. If the exclusion of oxygen is required, an inert dry gas must be used. The gas is dried by a condenser immersed in the storage vessel (Figure 5, 50).

Figure 5 shows the storage vessel from which the cooling fluid flows to the chamber. This vessel, 10, is carefully insulated against thermal losses, 30, 31. The circulation of the cooling fluid to the chamber and back to the storage vessel is achieved by a screw pump, 20, 23, 24, driven by an electric motor. To prevent the inflow of solid particles into the pump and into the temperature chamber a 24-mesh brass screen, 11, is fitted into the storage vessel.

The temperature in the chamber is measured by the copper-constantan thermocouple, one point of which is soldered directly into the chamber (Figure 3, 13); the other end is immersed in an ice bath or in the cooling fluid in the storage vessel (Figure 5, 130). In the latter case, the temperature of the cooling fluid should be measured by a low-temperature thermometer (Figure 5, 40) (*5*, *8*, *10*, *12*).

For cooling fluids from room temperatures to −55° C., mixtures of ice and different salts are used. For temperatures down to −100° C. solid carbon dioxide with acetone, ether, or other organic solvents is used. A temperature of −180° C. is produced by liquid air. This must be handled very carefully and poured slowly into the storage vessel (*11*, *17*).

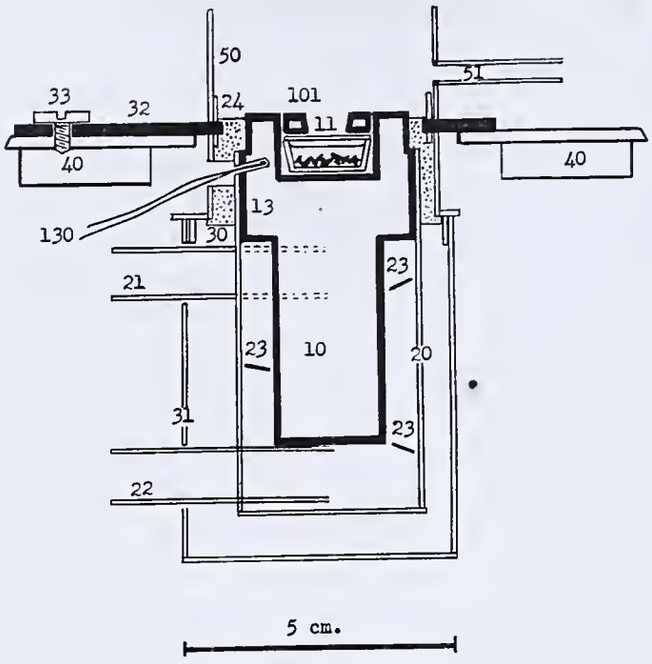

FIGURE 3

The chamber temperature is regulated within definite ranges by changing the speed of the circulating fluid.

A constant temperature of the sample is reached in about 12 minutes and is 2 to 5 per cent higher than the temperature in the storage vessel.

By using hot water or oil this microchamber can also be used for higher than room temperatures. An immersion heater is immersed in the storage vessel for this purpose (*17*).

Sometimes it is necessary to observe the sample not only under the influence of low temperatures but also under the influence of different pressures (high pressure or vacuum). Figure 4 shows the microchamber for high pressure and vacuum application. The chamber is covered with a pressure-resistant glass, 12. Pressures to 75 atmospheres (1100 pounds per sq. inch) or vacuum to the vapor pressure of the sample or of the solvent of the sample can be applied through the tube, 140, 14.

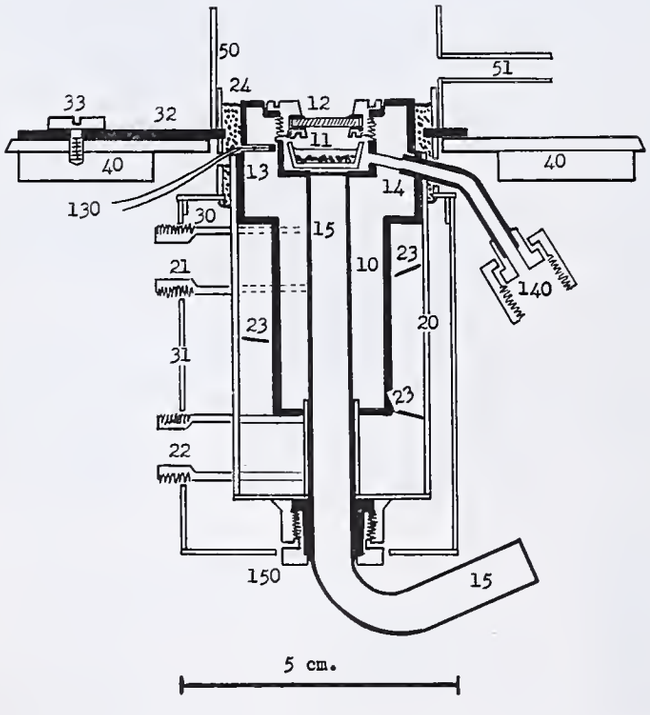

FIGURE 4

The temperature and the pressure can be read simultaneously by using a camera lucida (*6*, *18*).

This microchamber was built especially for investigations of the behavior of unsaturated fatty acids under the influence of low temperatures (*3*, *4*, *16*). It can also be used for determining melting points of low freezing liquids and other physical chemical constants.

Summary

A microchamber for observing chemical and biological work at low temperatures and varying pressures is described. It can also be used for determining such physical chemical constants as melting point, etc. The sample can be observed through the microscope. A temperature range from +80° to −180° C. is obtained by circulating liquids—hot water or oil, cold water, ice-salt mixtures, dry ice–acetone mixtures, and liquid air. The microchamber can be attached to any microscope.

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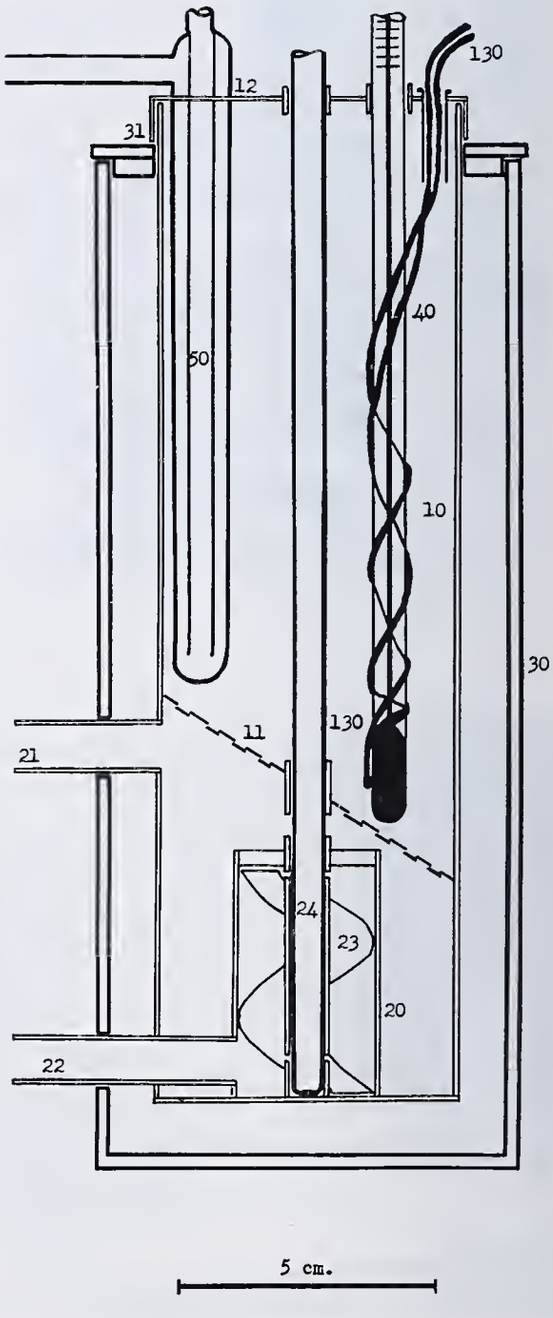

FIGURE 5

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Determination of Lead in Biological Material

A Polarographic Method

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POLAROGRAPHIC methods for the determination of lead in biological material have been described by Shikata (*12*), Yoshida (*15*), Hamamoto (*7*), Teisinger (*13*), and Forche (*6*). Several of these methods can be applied only to specific materials (*6*, *7*, *12*, *13*), others lack sensitivity and accuracy (*6*, *15*), and none is entirely satisfactory for general use.

An effort to overcome some of these difficulties led to the development of a procedure for the direct electrolytic deposition of lead from solutions of ashed material (*2*) and made possible the quantitative isolation of lead in small volumes of test solution which can be polarized satisfactorily. A method based on this electrolytic technique has proved to be applicable to every type and condition of sample likely to be encountered. It is presented here not as an improvement over the best of the chemical and spectrographic methods in current use but as an interesting and successful application of a new analytical tool. In addition to the electrolytic means of isolating and concentrating the lead, a method is also given for the separation of the lead with dithizone followed by electrolytic concentration, if necessary. The latter provides for the accurate analysis of certain samples (feces and individual or mixed foods) with greater speed and convenience.

Polarographic Technique

Types of apparatus and the theory of analysis by the use of the dropping-mercury cathode have been fully covered by Hohn (*8*), Kolthoff and Lingane (*10*), and Walkley (*14*), among others; therefore we need concern ourselves only with the practical applications of the method. Although the operation of the equipment is relatively simple, a number of factors must be considered if the end result is to be reliable. Purity of reagents and precision in chemical processes are obviously required, and it should also be recognized that the current-voltage curves are markedly affected by the size of the mercury drop, its dropping rate, the temperature, and by the composition of the test solution.

Several of these difficulties may be overcome by rigid standardization of technique, but, despite continual and tedious experimentation, standard procedures can scarcely be devised which will permit the use of a single working curve for every type of sample that may be encountered. Forche (*6*) has shown, however, that the employment of "internal standards", an adaptation from spectrographic analysis, results in a marked stability of the working curves. He (*5*, *6*) introduced a known amount of cadmium into his standards and samples, and then employed the ratio of the lengths of the lead and cadmium steps as a measure of the lead concentration. After the procedure had been standardized, the ratios were independent of variations in the dropping rate or size of the mercury drop, and the composition or viscosity of the test solution. That the step ratio is also independent of the temperature is indicated in Table I, and moreover the ratios are not affected by recorder sensitivities.

Only two curves need be developed to fit any condition encountered in practice (Figures 1 and 2). They were obtained by polarizing 2-ml. portions of solutions containing known amounts of lead and cadmium. The total volumes and types of solutions used, the amount of cadmium added, and the polarizing conditions have been indicated on the charts. The current-voltage curves from −0.3 to −0.9 volt were obtained with the Leeds & Northrup Chemograph which employs a Micromax ink recorder instead of the usual photographic tracing of the galvanometer swing.

Table I. Effect of Temperature on Lead-Cadmium Step Ratio

(Drop rate = 10 drops in 30 seconds)

Temperature °C.	Pb Step *Mm.*	Cd Step *Mm.*	Pb/Cd Ratio
24	16.8	31.8	0.528
30	19.2	36.3	0.529
35	20.1	38.2	0.526
40	22.2	42.7	0.520
		Av.	0.526

Procedure

Preparation of Samples. The biological material was ashed at 500° C. as described in previous papers (*3*, *4*, *9*). The ashed materials were brought into solution by addition of nitric or hydrochloric acid and distilled water and the solutions were rinsed into glass-stoppered Pyrex cylinders or volumetric flasks, the volumes being adjusted with distilled water as dictated by experience.

Electrolytic Isolation and Concentration. An aliquot or the entire sample, consisting of 5 to 20 grams of blood or solid tissue, 100 to 250 ml. of urine, 0.1 to 0.15 gram of the ash of feces, or $^1/_{20}$ of a day's mixed food, is placed in a 50-ml. Pyrex beaker and the lead is collected on a platinum gauze electrode, 9 mm. in diameter and 25 mm. high, by a method described elsewhere (*2*). The electrode is washed with hydroquinone solution (0.1 gram in 100 ml. of distilled water, and made just alkaline with ammonium hydroxide immediately before use), is dried, and the lead is stripped from it into 2 ml. of stripping solution (10 per cent tartaric acid, 0.08 *N* nitric acid, 6 micrograms of cadmium per ml.) in the cell over the anode pool of mercury. This cell is so constructed that when the gauze cylinder is inserted it is completely covered by the solution in the cell. [In dealing with very minute amounts of lead (1 to 10 micrograms) it is desirable to strip the lead into 1 ml. of solution, and for this purpose a plati-

num gauze electrode 6.5 to 7 mm. in diameter and only 15 mm. high is employed.] Nitrogen (freed from oxygen by bubbling through ammoniacal cuprous chloride solution and then through dilute sulfuric acid) is passed slowly through the solution for 5 minutes to remove dissolved oxygen, and the solution is polarized from −0.3 to −0.9 volt at a suitable recorder sensitivity. The "step ratio" (Pb/Cd) is then obtained and the quantity of lead (corrected for the volume of solution polarized) is read from Figure 1.

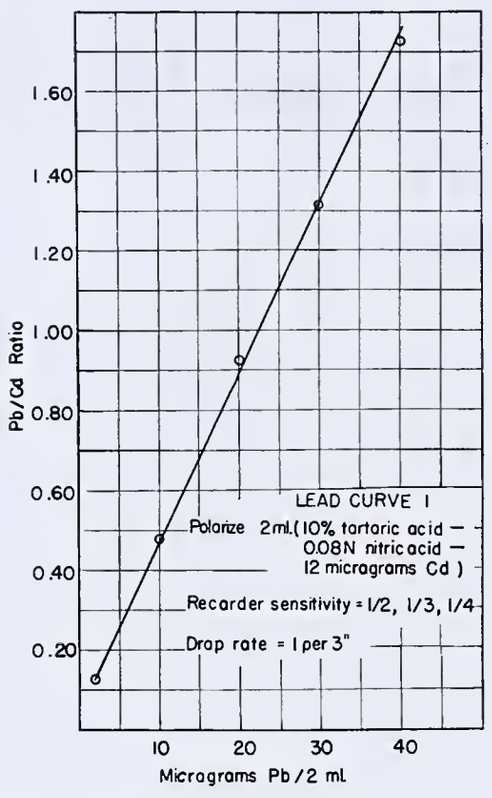

FIGURE 1. LEAD CURVE

In dealing with feces, individual or mixed foods, or other samples containing relatively large amounts of copper and iron, electrolysis in the presence of ammonium citrate and potassium cyanide is employed for one hour (*2*), or a double electrolytic technique may be used, consisting of an initial half hour's electrolysis in the presence of ammonium citrate followed by an hour's electrolysis in the presence of potassium cyanide (*2*).

DITHIZONE ISOLATION AND ELECTROLYTIC CONCENTRATION. As before, either a suitable aliquot or the entire sample is used and the lead is first removed by means of dithizone (0.03 gram in 1000 ml. of chloroform), preferably in the presence of citrate and cyanide (*1*). After the dithizone extract has been washed with 50 ml. of distilled water, the lead is shaken into 20 to 40 ml. of dilute nitric acid (1 ml. of nitric acid, specific gravity 1.40, in 100 ml. of distilled water). If the color of the dithizone extract has indicated that the amount of lead is low (less than 40 micrograms), the acid extract is placed in a 50-ml. beaker, 2 ml. of deleaded ammonium citrate (40 grams of citric acid per 100 ml.) are added, the solution is made just alkaline to phenol red, and the lead is electrolyzed onto the 9 × 25 mm. gauze electrode for 30 minutes. After the electrode has been dried (washing of the electrode is not necessary), the procedure is the same as that described above. In case less than 10 micrograms of lead is expected or indicated by the dithizone extract, the 7 × 15 mm. electrode is used, so as to permit stripping of the lead into 1 ml. of solution.

When more than 40 micrograms is present, analysis may be accelerated by eliminating the electrolytic step. If the lead content is sufficiently high, the acid washing of the extract may be polarized immediately after the addition of the proper amount of cadmium to serve as the internal standard, or the lead may be concentrated in a smaller volume of test solution. In the latter case the acid extract is evaporated to small volume (3 to 5 ml.); the solution is made just acid to phenol red; 1 ml. of a cadmium solution (90 micrograms of cadmium) is added; and the volume is made up to 10 ml. in a glass-stoppered Pyrex cylinder. Two milliliters of this solution are polarized at recorder sensitivities indicated on Figure 2.

TABLE II. RECOVERIES OF LEAD ADDED TO SYNTHETIC URINE ASH

(Samples correspond to 100 ml. of urine)

Lead Added	Lead Found: Polarographic Method: Electrolytic isolation and concentration		Lead Found: Polarographic Method: Combined extraction and electrolysis		Lead Found: Dithizone method (*1*)	
Micrograms	*Micrograms*		*Micrograms*		*Micrograms*	
0	0.0	0.0	0.0	0.0	0.2	0.2
2	2.2	2.5	2.8	2.7	2.3	2.3
16	16.5	15.3	16.0	15.0	16.3	17.3
42	39.0	39.5	40.0	40.0	40.8	41.3
80	80.0	78.0	80.0	82.0	79.0	79.0

TABLE III. COMPARATIVE RESULTS ON COMMON BIOLOGICAL MATERIAL

Material	Lead by Polarographic Method: Electrolytic isolation and concentration	Lead by Polarographic Method: Combined extraction and electrolysis	Lead by Dithizone Method (*1*)	Lead by Spectrographic Method (*4*)
	Mg.	*Mg.*	*Mg.*	*Mg.*
Feces[a]	0.55	0.56	0.58	0.58
	0.29	0.29	0.30	0.32
Food[b]	0.21	0.20	0.22	0.22
	0.44	0.39	0.43	0.41
	Mg./l.	*Mg./l.*	*Mg./l.*	*Mg./l.*
Urine	0.035	0.036	0.038	0.043
	0.170	0.160	0.170	0.170
	0.086	0.082	0.087	0.087
	Mg./100 g.	*Mg./100 g.*	*Mg./100 g.*	*Mg./100 g.*
Blood	0.065	...	0.070	0.065
	0.080	...	0.095	0.085
	...	0.071	0.071	0.075
	...	0.060	0.064	0.070

[a] Twenty-four hour samples.
[b] Mixed food samples duplicating that eaten by experimental subject in 24 hours.

Results

In Table II are listed recoveries of lead added to portions of a synthetic urine ash solution, in which 5 ml. are equivalent to the ash of 100 ml. of normal urine (*3*). For purposes of comparison, results obtained for the same samples by the photometric dithizone method (*1*) have also been listed in this table. Results obtained on a number of random samples by the polarographic, spectrographic, and dithizone methods are recorded in Table III. Except in the case of the blood samples, sufficient material was available to provide suitable aliquots for each of the four methods. The tissues listed in Table IV were from a person with a known lead exposure,

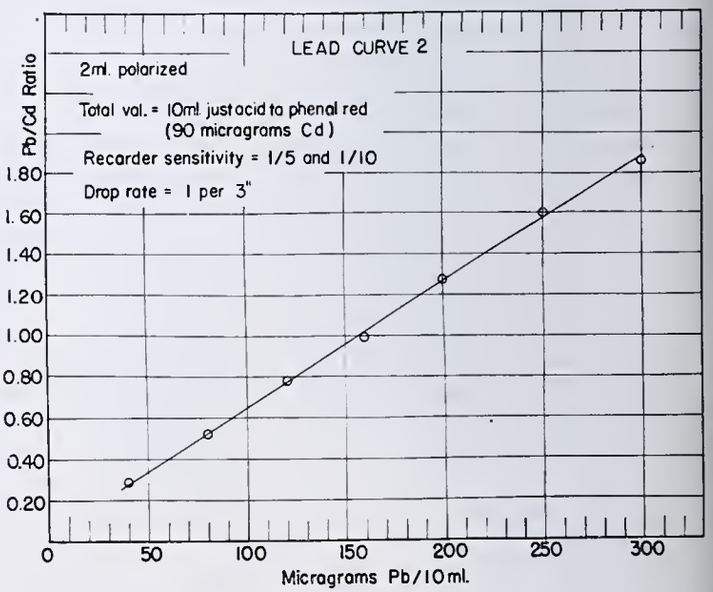

FIGURE 2. LEAD CURVE

which, however, was unrelated to his death. The results by four types of procedures are given when the sample was large enough.

Discussion

From the standpoint of accuracy and convenience, either polarographic method compares favorably with the dithizone and spectrographic methods and its use for routine analytical purposes is fully justified. Results tend to be slightly lower than with the other methods, but the difference is insignificant. For certain materials the electrolytic technique is more time-consuming than the combination of extraction and electrolysis, but the former is so simple and requires so little attention that it can be carried out simultaneously with other laboratory work. In addition, its manipulative steps are so distinctly different that its use gives additional confirmation of results obtained by other methods (*1*, *3*, *4*, *9*).

The extraction-electrolysis modification removes practically all extraneous metals, is highly flexible, and lends itself well to a number of obvious variations which greatly enhance its practical value. Frequently enough material is available to permit the use of aliquots yielding lead in sufficient amounts to permit direct polarization of the acid washings of the dithizone extracts.

Attempts to polarize solutions of ashed material directly did not prove entirely satisfactory. In the case of urine samples satisfactory cathodic curves were obtained when the solutions were made just acid to prevent the precipitation of phosphates, but the dilution required to keep all salts in solution reduced the sensitivity of the method to such an extent as to limit its practical value (*c*, Figure 3). In addition, the presence of other reducible metals is troublesome; traces of lead can be determined only if the quantities of iron, copper, and bismuth in the solutions are definitely limited, while mere traces of stannous tin, trivalent cobalt, and thallous ions interfere because of the practical coincidence, in neutral or acid solution, of their half-wave potentials with that of lead (*11*).

In addition to the possible masking effects of the iron steps, cathodic curves at high recorder sensitivities for solutions containing ferric iron are very poor, usually because of a high limiting current (*d*, Figure 3). Ferric iron in the test solution reacted in some manner with the mercury of the anode pool (possibly by oxidation of the mercury) to give a smudgy material at the interface, which spread rapidly through the solution as soon as nitrogen was passed through it. This precipitate developed with as little as 100 micrograms of Fe^{+++} per ml. of solution and increased in amount with the quantity of the iron. Spectrograms of test solutions containing this precipitate and of the clear solution after settling of the deposit showed that the precipitate was due to some unidentified mercury compound, either occurring in combination with ferric ions or precipitated by them.

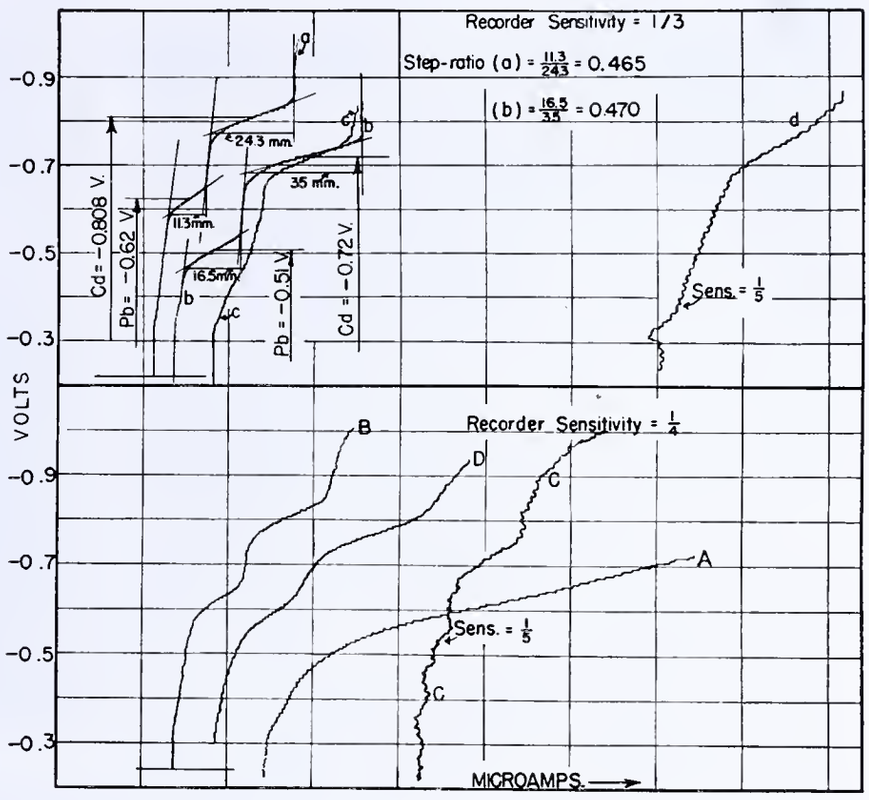

FIGURE 3. CURVES FOR SOLUTIONS OF ASHED MATERIAL

A, *B*, *C*, *D*. Equal aliquots of same urine
A. Electrode not washed. Base, tartaric acid–nitric acid
B. Electrode washed with alkaline hydroquinone solution. Base, tartaric acid–nitric acid
C. Same as *B*, except base potassium chloride–hydrochloric acid solution
D. Electrode washed with hydroxylamine–hydrochloric acid solution. Base same as *A*

In most cases, therefore, the advantage of isolating the lead electrolytically or by extraction with dithizone is obvious, while the necessity for concentrating lead becomes apparent from consideration of comparative ultimate sensitivities. Practical considerations limit the volume of the test solution to 1 ml., in which as little as 1 microgram of lead can be detected by the polarograph. This is a disadvantage, as compared with spectrographic and dithizone methods. (One microgram of lead in 10 ml. of solution can be estimated spectrographically by using only 0.2-ml. aliquots, *3*, *4*. The ultimate sensitivity of the dithizone method seems to be limited by the magnitude of the rack blank. In one procedure the latter has been reduced to 0.1 microgram, *1*, thereby making it possible to estimate a fraction of a microgram of lead in any volume of solution which can be handled in the rack equipment.) This limitation in performance on the part of the polarograph is not of practical significance, however, since very few occasions will arise when it will not be possible to isolate at least 1 microgram of lead.

Two stripping media—a 10 per cent tartaric acid–0.08 *N* nitric acid solution and a 0.1 *N* potassium chloride–0.1 *N* hydrochloric acid solution—were effective in dissolving lead from the electrodes, but the former is preferable, especially when only the electrolytic method of lead isolation and concentration is used. In order to obtain satisfactory polarograms by this method, it was found necessary to wash the elec-

TABLE IV. COMPARATIVE RESULTS ON HUMAN TISSUES[a]

Tissue	Weight	Lead by Spectrographic Method (*4*)	Lead by Dithizone Method (*1*)	Lead by Polarographic Method: Electrolytic isolation and concentration	Lead by Polarographic Method: Combined extraction and electrolysis
	Grams	*Mg. per 100 grams*			
Spleen	28.4	1.05	1.18	1.12	1.10
Heart	30.1	0.050	0.032	0.035	0.045
Suprarenal	6.0	0.24	...	...	0.20
Stomach	26.5	0.085	0.095	0.080	0.070
Lung	49.0	0.39	0.40	0.36	0.35
Muscle	29.0	0.045	0.03	0.025	0.035
Thyroid	4.5	0.62	...	0.57	...
Small intestine	18.5	0.070	0.060	0.06	0.065
Rib bone	6.5	16.85	17.7	17.1	18.2

[a] In most cases aliquots employed represent very small quantities of material, so that differences between results were often no more than 1 microgram.

trodes before stripping the lead from them. A number of wash solutions were tried; water was found to cause a slight loss of lead, and weak solutions of sodium sulfite and sodium hyposulfite were not satisfactory. A dilute solution of hydroxylamine hydrochloride did not affect the quantitative recovery of lead and the polarograms were greatly improved, but slightly low results were always obtained because of an artificial lengthening of the cadmium step. The use of hydroquinone solution, however, eliminated this difficulty and satisfactory polarograms were consistently obtained when the electrodes thus washed were stripped in the tartaric acid–nitric acid base. When the electrodes were stripped in the hydrochloric acid–potassium chloride base, occasional curves (such as *C* in Figure 3) were obtained which were too irregular to permit accurate measurement of the steps. Acid and alkaline potassium tartrate media were also tried but these did not prove satisfactory, because of troublesome precipitates, high limiting currents, or fusion of the lead and cadmium steps. The latter reason no doubt accounts for Forche's failure to use the step-ratio procedure in his method for lead determination in blood, even though he first suggested its use for analytical purposes (*6*).

A number of the effects just discussed are illustrated by the polarograms reproduced in the lower section of Figure 3. In addition at *a* and *b*, respectively, of the upper section of the figure, polarograms for equal amounts of lead added to the tartaric acid–nitric acid and the potassium chloride–hydrochloric acid bases are reproduced. The method of slopes used to obtain the step ratios, as well as the shift of the half-wave potentials for the lead and cadmium steps due to the change in composition of the bases, have been indicated for these polarograms. The sensitivity is somewhat less in the tartaric acid–nitric base, but this is compensated for by the improvement in the curves, permitting more certain measurements of the steps. At *c* and *d*, respectively, are illustrated cathodic curves for the direct polarization of solutions of ashed urine and feces samples. The aliquots chosen were concentrated to 10 ml. after being made just acid to prevent the precipitation of phosphates, and although 8 and 12 micrograms of lead were present, respectively, neither *c* nor *d* shows the presence of lead. In the case of *d*, a high limiting current, as indicated by the shift of the curve to the right, is attributed to the presence of ferric ion, since the characteristic precipitate described above formed in the test solution.

In practice the direct electrolytic isolation and concentration of lead from prepared samples of biological material has been found effective except in occasional samples which contained bismuth and large amounts of copper. In many cases these interferences may be overcome by using smaller aliquots or by diluting the solution to be polarized. If distinct steps are not obtained by this procedure, it is best to repeat the analysis on a fresh aliquot by the modified method. Bismuth, if present, can be extracted with dithizone at pH 2 as described in previous papers (*1*, *9*). The possibility of isolating and concentrating bismuth electrolytically suggests that a similar method might be developed for its polarographic determination. In this case, as the half-wave potential of bismuth is considerably below that of lead, relatively large amounts of the latter should not interfere.

Summary

A polarographic method is described for the estimation of lead in biological material, based on electrolytic isolation and concentration of lead followed by stripping into very small volumes of solution. Two methods of concentration are presented: (1) a purely electrolytic technique, and (2) an initial dithizone isolation, followed by electrolytic concentration when very small amounts of lead are present.

An adaptation from spectrographic analysis of the internal standard principal permits the derivation of stable working curves, which are not affected by variations in temperature, drop size or rate, viscosity or composition of the solution to be polarized, or by changes in the recorder sensitivity used.

The accuracy and practical sensitivity of the method approach those of the spectrographic and dithizone methods.

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Volumetric Tubes for Small Volumes

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THE appearance of the description of a volumetric flask by Caley (*1*) prompts the publication of other modifications which have been used in this laboratory.

Figure 1, *A*, shows a form superficially similar to the old Folin sugar tube. The contents of the bulb are mixed in the same manner as in Caley's flask, except that the tube cannot be brought quite to the horizontal position; some solution is

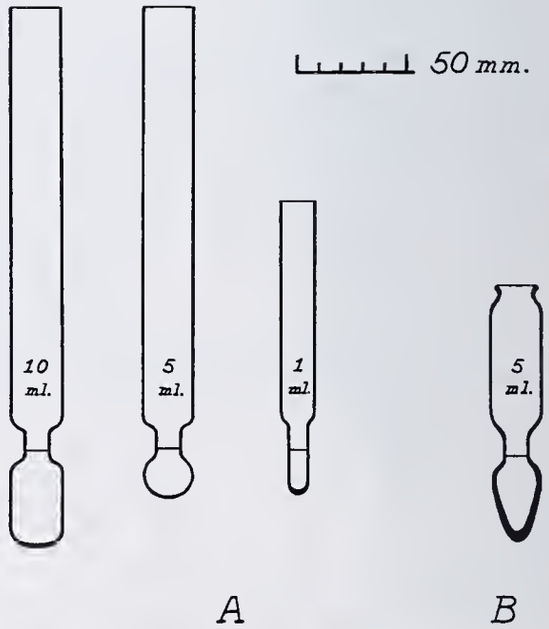

Figure 1. Volumetric Tubes

left in the bulb, and it may be necessary to repeat the operation a few more times. However, these volumetric tubes are more easily handled and are more conveniently held in a standard test tube rack than a flask having a large bulb, and they occupy less space and are less subject to breakage when stored in a drawer, stacked at the sink, or placed in a cylinder for washing.

Both Caley's flask and the author's tubes offer several advantages over the conventional volumetric flask. When liquid is added by means of a pipet the tip is held on the shoulder above the constriction, thus decreasing the size of the drop held by capillary action between the tip of the pipet and the wall of the vessel and greatly shortening the path of drainage into the calibrated bulb. It is often possible to conduct several or all operations in the volumetric flask or tube, even when the volume greatly exceeds that for which it is calibrated. Relatively large volumes can be boiled without loss by splashing. In boiling off one solvent preparatory to substituting another, the deep mass of solvent vapor remaining in the vessel protects the dry residue from contact with the air. The tubes can be immersed to any desired depth in the steam bath to control the rate of boiling and of reflux. The deep narrow top part can accommodate a simple reflux condenser (without ground joint or cork or rubber stopper), making it possible to conduct prolonged reactions at boiling temperature. In both the flask and the tubes, avoidance of contact of solution with a stopper eliminates not only the error mentioned by Caley but also the loss of solution which may result from sudden expansion of vapor from a volatile solvent.

With some sacrifice of advantages of the deeper tubes, a shorter volumetric tube can be used with the 50-ml. centrifuge cup. Such a tube is shown in Figure 1, *B*. In this tube a precipitate can be thrown down, washed, dissolved, and made up accurately to a given volume without transferring the precipitate from the tube in which it is formed.

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Microscopy of the Amino Acids and Their Compounds

Silver Salts

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THIS paper reports a continuation of a systematic investigation of the microscopy of amino acids and their compounds in the effort to characterize as many as possible with respect to their crystalline habits and optical crystallography. Previous papers of the series have dealt with the amino acid phosphotungstates and phosphomolybdates (*2*), picrates and flavianates (*3*), copper salts (*4*), and picrolonates (*6*). In this paper, data relative to silver salts of the available amino acids are reported.

Silver salts of amino acids have been prepared rather frequently for purposes of their isolation as well as characterization. Abderhalden (*1*) lists the preparation of the salts of glycine, leucine, isoleucine, aspartic acid, glutamic acid, arginine, cystine, tyrosine, tryptophane, and histidine. Kossel and Kutscher (*7*) utilized the insolubility of the silver salts of arginine and histidine as a method for isolation of those amino acids, a procedure which has undergone many modifications since the original publication. Kutscher (*8*) proposed a similar utilization of the silver salts of aspartic and glutamic acids for the isolation of these compounds. Dakin (*5*) utilized silver salt formation in the isolation of hydroxyglutamic acid.

Since the purpose of this study is the development of rapid and easy methods for identifying the amino acids and their derivatives, no attempt has been made to study completely the crystallography of the salts. As a general rule, those properties which are immediately available for study, such as extinction position, sign of elongation, and the two prominent refractive indices, have been determined. The refractive indices of the silver salts were not determined because of the very great difficulty encountered from blackening of the crystal by light and contamination by silver nitrate which made such data unreliable. The other properties listed were studied. A crystal of solid amino acid was dissolved by warming it in a drop of water on the microscope slide, producing a rather concentrated solution, to which was added a small crystal of solid silver nitrate. The silver salts usually appeared shortly. Their form was improved in most cases by recrystallization. Special cases involving deviations from this simple procedure are given in the description of the compounds below. Satisfactory crystalline compounds were obtained with alanine, arginine, aspartic acid, dibromotyrosine, dichlorotyrosine, diiodotyrosine, glutamic acid, glycine, histidine, hydroxyvaline, isoleucine, leucine, norleucine, norvaline, phenylalanine, hydroxyproline, serine, tryptophane, tyrosine, and valine.

The following amino acids failed to give silver salts: cystine, lysine, cysteine, isoserine, proline, and methionine.

Results

The following silver salts were successfully prepared:

Alanine. The silver salt of alanine formed flat transparent acicular prisms with rather square ends (Figure 1, upper left). These formed slowly along the edge of the drop and were anisotropic with parallel extinction, and elongation (−).

Arginine. The silver salt of arginine formed in rather long transparent acicular prisms occurring singly, twinned, and in groups (Figure 1, upper center). Crystals were anisotropic with parallel extinction, and elongation (−).

Aspartic Acid. The silver salt of aspartic acid usually formed long prisms along the edge of the drop (Figure 1, upper right). These were anisotropic with parallel extinction, and elongation (−).

Dibromotyrosine: The silver salt of dibromotyrosine formed initially as a heavy precipitate of fine needles which grew into tufts of short prisms (Figure 1, left center). These were anisotropic with parallel extinction, and elongation (+).

Dichlorotyrosine. The silver salt of dichlorotyrosine formed initially a few sheaves of small needles which later grew into dark

FIGURE 1. AMINO ACID SILVER SALTS, × 37 TO 160

Upper. Alanine, arginine, aspartic acid. Center. Dibromotyrosine, diiodotyrosine, glycine. Lower. Leucine, serine, tryptophane

tufts of prisms. They were anisotropic with parallel extinction, and elongation (+).

DIIODOTYROSINE. The silver salt of diiodotyrosine formed a dense precipitate which grew into rosettes of fine needles. Dilute solutions allowed formation of fine acicular prisms which were used for optical examination (Figure 1, center). They were anisotropic with parallel extinction, and elongation (+).

GLUTAMIC ACID. The silver salt of glutamic acid formed chains of small rosettes along the edge of the drop, the size increasing considerably with time. They appeared dark colored, brown or black depending on the size which varied the opacity. They were anisotropic with parallel extinction, and elongation (−).

GLYCINE. The silver salt of glycine formed large amounts of both short and long acicular prisms along the edge of the drop. These gave the appearance of transparency (Figure 1, right center). They were anisotropic with parallel extinction, and elongation (−).

HISTIDINE. The silver salt of histidine was sufficiently insoluble to give good preparations, but only from moderately dilute solutions. Gentle heating of these for several minutes gave small prisms either singly or as inclined twins. From concentrated solutions, heavy dark branching dendrites were obtained. Both forms were anisotropic with parallel extinction, and elongation (−).

HYDROXYVALINE. The silver salt of hydroxyvaline formed long transparent acicular prisms along the edge of the drop, which tended to group in a parallel form in bundles. They were anisotropic with parallel extinction, and elongation (−).

ISOLEUCINE. The silver salt of isoleucine formed in sheaves and in single long transparent acicular prisms along the edge of the drop. They were anisotropic with parallel extinction, and elongation (−).

LEUCINE. The silver salt of leucine, upon slow evaporation, formed long thin transparent prisms in a random network (Figure 1, lower left). They were anisotropic with parallel extinction, and elongation (−).

NORLEUCINE. The silver salt of norleucine formed very slowly as fine transparent needles along the edge of the drop. These were anisotropic with parallel extinction, and elongation (−).

NORVALINE. The silver salt of norvaline formed very slowly as long flat acicular prisms with pointed ends. The crystals were transparent. They were anisotropic with parallel extinction, and elongation (−).

PHENYLALANINE. The silver salt of phenylalanine formed as sheaves and bundles of transparent acicular prisms along the edge of the drop. They were anisotropic with parallel extinction, and elongation (−).

HYDROXYPROLINE. The silver salt of hydroxyproline formed as sheaves of very fine acicular prisms along the edge of the drop as the evaporation proceeded to nearly dryness. They were anisotropic with parallel extinction, and elongation (−).

SERINE. The silver salt of serine formed flat prisms along the edge of the drop upon slow evaporation (Figure 1, lower center). They were anisotropic with parallel extinction, and elongation (−).

TRYPTOPHANE. The silver salt of tryptophane appeared slowly as sheaves and rosettes of fine transparent acicular prisms (Figure 1, lower right). These were anisotropic with parallel extinction, and elongation (−).

TYROSINE. The silver salt of tyrosine was formed as tufts of fine transparent acicular prisms which appeared dark with transmitted light. They were anisotropic with parallel extinction, and elongation (+).

VALINE. The silver salt of valine was formed slowly as long pointed transparent acicular prisms occurring singly and in sheaves. They were anisotropic with parallel extinction, and elongation (−).

The fact that nearly all the silver salts of amino acids crystallize in the form of needles renders their use in identification somewhat less valuable than has been true of other reagents studied in this series. This deficiency is increased by the very great difficulty of obtaining refractive index data on the crystalline products. Because of the great similarity of many of the preparations, only nine of the more characteristic ones are shown in Figure 1. In addition to the crystals described, two amino acids reacted with silver nitrate—cysteine, which gave a red-colored gelatinous precipitate, and methionine, which gave an oily liquid. The crystals that may be considered characteristic for identification purposes are those of diiodotyrosine, dibromotyrosine, glutamic acid, histidine, dichlorotyrosine, and tyrosine.

Tyrosine and its halogen derivatives showed positive elongation in contrast to all other preparations. In no case did sulfur-containing amino acids produce any crystals.

Acknowledgment

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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Chemical Factors in the Determination of Water in Insulating Oil

A New Electrical Method

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Consolidated Edison Company of New York, Inc., Brooklyn, N. Y.

The presence of small quantities of water in insulating oil seriously impairs its usefulness in electrical equipment. The need for an accurate as well as rapid method for its determination cannot be overemphasized. The present paper describes the experimental modifications of the combustion procedure directed toward lowering the hydrocarbon correction. The influence of temperature in the removal of the water from oil and the limitations of the Grignard procedure are experimentally demonstrated. An electrical method is briefly described and its application to a field determination of water is pointed out.

IN RECENT years several chemical procedures have been advanced which claim specificity for the estimation of water in organic liquids.

The Fischer method (*6*), worked on extensively in this country by Smith, Bryant, and Mitchell (*12*), depends on the oxidation of sulfur dioxide by iodine in the presence of water. The latter authors first worked out the estimation of water by acetyl chloride, which was adapted by Clark to the determination of water in transformer oil. Because of the color of an insulating oil and the probable presence of interfering substances in the original oil sample, it appears likely that in any chemical procedure the water first must be removed from the oil, as, for example, in the procedure described by the authors (*4*). The chief difficulties with the Fischer method and the acetyl chloride method in their application to oils are the estimation of peroxides in the former method and the estimation of volatile acidity in the latter. Two additional methods involve the use of α-naphthoxydichlorophosphine (*9*), which is not specific to the OH group of water, and of benzoic anhydride (*11*). The Grignard reagent has also been suggested by Larsen (*7*) as a possibility for determination of traces of water in oils.

In the method (*4*) which the authors have described, the volatile carbonaceous material and the free water, after being trapped out by the use of a Dewar flask containing solid carbon dioxide, were carried into a combustion furnace. The products of combustion and the free water were absorbed in microchemical absorption tubes and weighed as carbon dioxide and gross water. The net water was obtained by applying an empirical correction ratio of water to carbon dioxide.

The accuracy of the method depends on the use of the correct ratio of water to carbon dioxide and the weight of carbon dioxide obtained in each experiment. Some knowledge of the former was obtained by continuing the experiment for 1 liter of nitrogen after the water weight had fallen to a minimum. The latter factor was greatly reduced by a change in procedure which is described in this paper. The ratio adopted for the mineral oil type of transformer oil was 0.3 and for the noninflammable synthetic oil 0.2. Carbonaceous material vaporized from a mineral oil which would be retained by the solid–carbon dioxide trap would be expected theoretically to have a water–carbon dioxide ratio of between 0.2 and 0.5. The higher than theoretical ratio (0.1) for the synthetic noninflammable oils may be due to partially chlorinated compounds below $C_{2n}H_nCl_n$ or to organic impurities absorbed in the operation of the transformer.

Apparatus

The modifications in the apparatus were directed toward the reduction of the amount of carbonaceous material entering the combustion furnace. It was found that the magnitude of the correction was greatly reduced by means of an oil scrubber in the train where preferential solution of the oil vapors took place. The change in procedure for the determination of water in oil is apparent from a description of the new section of the apparatus as shown in Figure 1. This section replaces *D* and *F* in Figure 1 of (*4*). (In *5*, page 301, in the description of the apparatus, "cell *E*" should read "cell *D*").

One hundred milliliters of oil were introduced into *C* through the serum rubber stopper and stopcock *D* after the residual water in the train had been removed. Approximately 10 ml. of Apiezon oil (an oil of low volatility used in high vacuum practice) were also added to cell *B*. Purified nitrogen gas in small bubbles formed by the porous fritted disk, *A*, carried the water from the oil sample into the solid carbon dioxide trap, *E*. During this period, scrubber *B* was maintained at a temperature of 100° C. When the removal of water was complete, the Dewar flask was removed and the nitrogen was directed around the oil sample cell by means of the three-way stopcock *G*. In this manner the

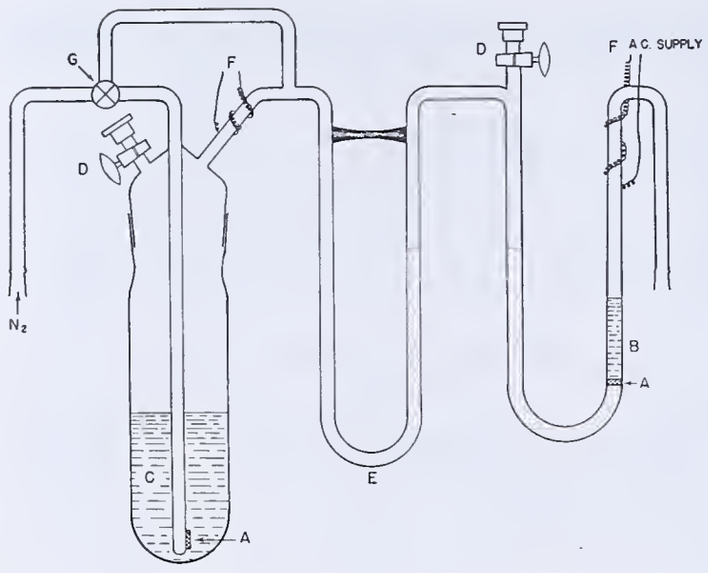

FIGURE 1. INTERCHANGEABLE APPARATUS FOR DETERMINING WATER IN INSULATING OIL

A. Porous fritted disks
B. Apiezon oil cell
C. Oil sample flask
D. Serum rubber stopper and stopcock
E. Dry ice trap
F. External spiral coiled heater
G. Three-way stopcock

trapped water in *E*, together with any volatile products, was carried through scrubber *B*, which was maintained at room temperature, into the combustion furnace. An external coiled heater, *F*, prevented frothing in either *B* or *C*. When the oil was of the halogenated type a spiral tube containing silver oxide maintained at a temperature of 400° C. was put in place at the exit end of the combustion furance. As is shown below, the temperature of the oil sample must be considered in connection with the removal of water. A safe maximum temperature would appear to be the average operating temperature of the oil. One may also be guided by the condition of the oil as revealed by chemical tests. Unless otherwise stated, a new mineral oil or the average nonflammable synthetic oil was heated to 105° C. during the passage of 2 liters of nitrogen, whereas for a used mineral oil the passage of 5 liters of nitrogen was carried out at 50° C. or at room temperature.

Efficiency of Apiezon Scrubber in Diminishing Carbon Dioxide Correction

In Table I is illustrated the reduction of the carbon dioxide correction by the use of the Apiezon-oil scrubber. When the oil scrubber was maintained at an elevated temperature, it was apparently ineffective in retaining carbonaceous material (experiments 3 and 3A). However, when the scrubber was maintained at room temperature it lowered greatly the quantity of oil vapor entering the combustion furnace. The adopted carbon dioxide–water correction apparently would have enabled one to obtain the correct results (experiments 3, 3A, A-16, and A-1) without the scrubber even where large amounts of carbon dioxide were obtained. The correction due to the solubility of water in the Apiezon oil is negligible, as was indicated by continuing the experiment until an additional liter of nitrogen had passed through the train (experiment 3A).

TABLE I. EFFICIENCY OF APIEZON SCRUBBER IN DIMINISHING THE CARBON DIOXIDE CORRECTION

Expt. No.	Oil Type	Temperature Oil scrubber	Temperature Oil sample	H_2O	CO_2	Gross H_2O	Gross CO_2	Net, H_2O
		° C.	° C.	*Mg.*	*Mg.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
3	Used 10C[a]	25	125	3.58	1.35	33	13	29
3A	Used 10C	110	125	4.75	8.00	62	105	31
3A (continued)	1 additional liter N_2	...	...	0.14	0.43	...	...	..
A-15	New 10C	Absent	25	3.34	5.80	108	187	52
A-15-A	New 10C	25	25	2.14	0.91	50	21	43
A-1	N. S. O.[b]	Absent	125	11.57	9.55	116	96	97
A-16	N. S. O.	25	125	3.87	0.58	102	15	99

[a] Mineral oil type of transformer oil.
[b] Nonflammable synthetic oil.

TABLE II. EFFECT OF TIME OF HEATING AND TEMPERATURE ON REMOVAL OF WATER FROM SAMPLE 6 (10C OIL)[a]

Expt. No.	Oil Sample Temperature	Weight of Sample	N_2	Gross H_2O	Gross CO_2	Net, H_2O
	° C.	*Grams*	*Liters*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	125	56	1	59	26	51
2	125	87	1.5	99	31	90
3	25	87	4	29	20	23
4	25	87	17	34	17	29
4 (continued)	125	..	4 additional	112	17	107
4 (continued)	125	..	18 additional	34	11	31
			Total water found in Expt. 4	167		

[a] Scrubber of Apiezon oil at room temperature.

Effect of Temperature on Removal of Water from Oil

The experimental results on many oil samples indicated that if an oil sample were heated while the water was being removed into the solid carbon dioxide trap high results were obtained which depended in magnitude on the time of heating. This effect was more evident in the mineral oil than in the nonflammable synthetic oil. Furthermore, the used transformer oils exhibited this effect to a greater degree than the new oils. In Table II, the results on a sample of used transformer oil (mineral oil type) are given. When experiments 1 and 2 are compared with experiment 3 (Table II, last column) the magnitude of the error involved is clearly illustrated. Thermal decomposition of oxygenated compounds was responsible for the formation of the additional water, although the residual dissolved oxygen gas in the sample might have played a minor part. In experiment 4 after 17 liters of nitrogen had passed through the oil sample at room temperature a very large increase in water content occurred when the temperature of the oil sample was raised to 125° C. Further heating yielded comparatively little additional water, indicating that oxygenated compounds were present in a limited amount. The completeness of removal of water at room temperature was indicated by the fact that the 4-liter run (experiment 3) yielded very little less than the 17-liter run (experiment 4). Thus by continued heating at 125° C. for a period involving the passage of 22 liters of nitrogen, an oil sample actually containing 30 p. p. m. of water was shown to behave as if 165 p. p. m. of water were present.

Behavior of Grignard Reagent in Determination of Water in Oil

The application of Grignard reagent to the determination of water in oils showed that after making allowance for the acid content determined on a duplicate oil sample the residual active hydrogen content of the oil sample is too large to be neglected. The term "residual active hydrogen" may be conveniently applied to the active hydrogen other than that of water and acids. Alcohols, amines, phenols, and acids too weak to be titrated may be considered to be the source of the residual active hydrogen.

Table III. Comparison of Results of Grignard and Combustion Methods

(Single oil sample at 25° and 125° C.)

Temperature of Oil Sample ° C.		Neutralization Number	Grignard Methane Evolved Found	Grignard Methane Evolved Corr.[b]	Grignard Calculated[a] Water	Combustion Water	Combustion Equivalent methane
			P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
25		0.36	226[c]	123	69	16	21
125	Distillate	0.04	95	67	38	..	..
	Residue	0.31	175	92	52	..	..
	Total			159	90	30	54

[a] $1H_2O = 2CH_4$. Acidity deducted using relation $1(H) = 1CH_4$.
[b] Blank deducted. Acidity deducted using relation $1(H) = 1CH_4$.
[c] Grignard reaction carried out in a mixture of 30% ether and 70% 10C oil.

It was first observed that the character of the oil sample (petroleum origin or chlorinated aromatic) influenced greatly the yield of the reaction. This phenomenon has been described by Hibbert and co-workers (*8*). It could be minimized by maintaining a large ratio of isoamyl ether to the oil sample. An attempt was made to analyze with the Grignard reagent the two fractions formed in the combustion procedure. The material which was condensed in the solid carbon dioxide trap (cell 2) is referred to in Table III as the distillate and that which remained in the oil cell (cell 1) as the residue. In a separate experiment, the acidity of the distillate and residue was determined by titration by means of a capillary syringe without removing either from the analytical train. The results bring out several points of interest. The large increase in water resulting from its removal from the oil sample at an elevated temperature (30 p. p. m. as compared to 16 p. p. m.) confirmed the results reported in Table II.

The major portion of the acid for this particular mineral oil sample was nonvolatile. In experiments with nonflammable synthetic oil carried out in a similar manner, the entire acid content remained in the residue. The residual active hydrogen appeared to a limited extent in the distillate (cf. 54 and 67 p. p. m. methane). After due allowance was made for the influence of the solvent and after the methane equivalent to the acid had been deducted, there was an apparent increase in the active hydrogen content of the oil sample as a result of heating to 125° C. (cf. 123 with 67 + 92 = 159 p. p. m. methane). This discrepancy (159 − 123 = 36 p. p. m.) was in good agreement with the difference between the calculated methane from the water obtained at 125° and 25° C. (54 − 21 = 33 p. p. m.). Except for the possibility of oxidation at the elevated temperature due to dissolved oxygen initially present in the oil sample, a probable explanation of the anomaly was related to the facts that ketones may enolize at the elevated temperature and that alcohols may split off water which under optimum conditions would yield two moles of methane as compared to a yield of but one mole of methane from the original alcohol.

It was concluded that the results of the Grignard test when expressed as evolved methane may be used only as an index of the stage of oxidation of an insulating oil. Based on experimental results with the microgravimetric procedure developed in this laboratory, it was considered extremely uncertain to ascribe the evolved methane to any particular chemical reactive group or compound after an acidity correction had been applied. Results of even greater uncertainty may be expected in the estimation of the Grignard added. The establishment of an oxygen balance by means of the results of the Grignard test did not appear to be of general application in the light of experimental work.

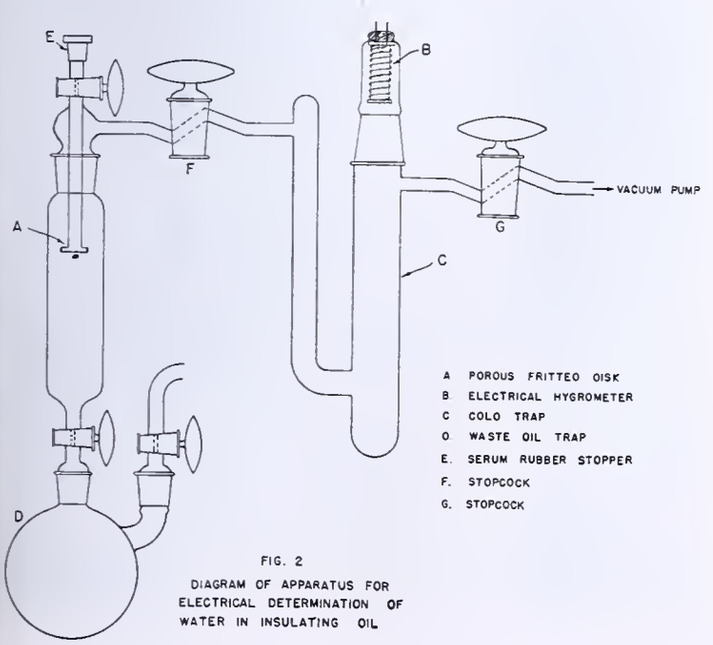

FIG. 2
DIAGRAM OF APPARATUS FOR ELECTRICAL DETERMINATION OF WATER IN INSULATING OIL

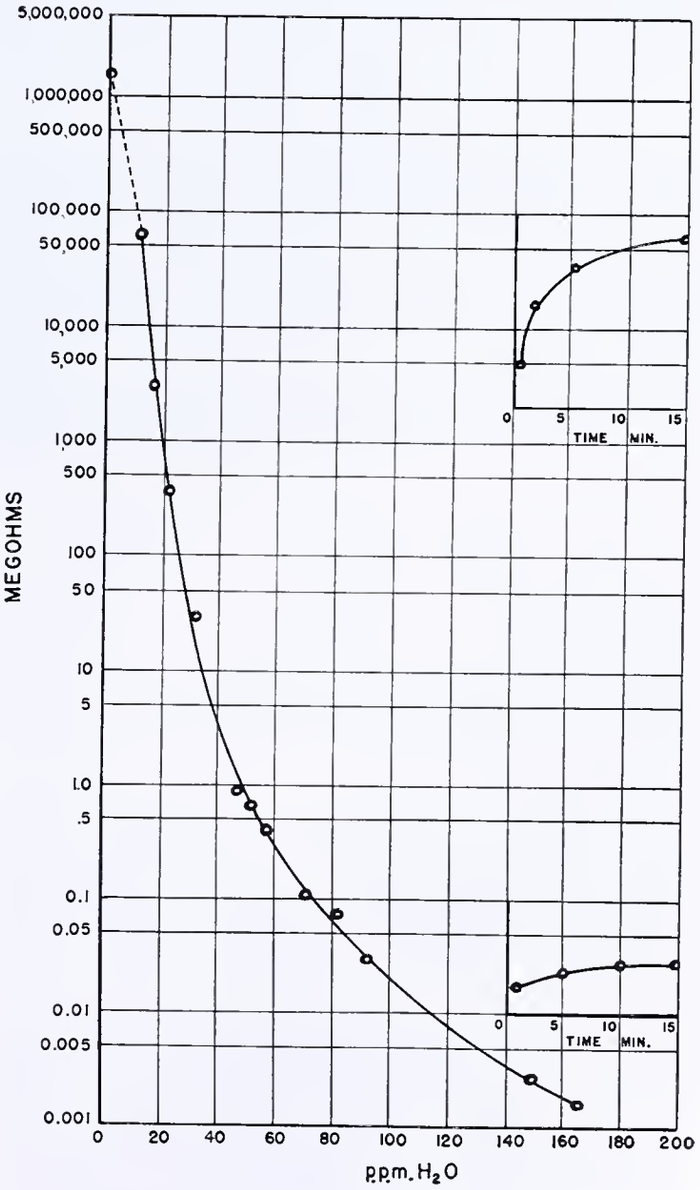

Figure 3. Relation between Electrical Resistance of Hygrometer and Water Content of Insulating Oils

Table IV. Solubility of Water in New Insulating Oils at Room Temperature

Expt No.	Oil Type	Temperature of Oil Sample	CO_2	H_2O Gross	H_2O Net	Clark's values at 25° C.
		° C.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
A-17	10C	25	7	46	44	...
A-15-a	10C	25	21	50	43	75
A-15-b	10C	125	32	57	47	...
A-16-a	N. S. O.	25	6	103	102	120 (Pyranol)
A-16-b	N. S. O.	125	15	102	99	...
A-12	5314[a]	125	7	54	52	70

[a] Mineral oil type of high-voltage cable oil.

Oil samples were prepared by shaking with an excess of water at 50° C. and cooling to room temperature. The saturated 10C oil was repeatedly decanted, the N. S. O. was repeatedly drawn off in a separatory funnel, and the 5314 oil was centrifuged.

Solubility of Water in Insulating Oils at Room Temperature

In a paper by Clark (*2*) the solubility of water in new Pyranol, transformer oil, and high-voltage cable oil was given. In connection with checking the precision of analytical trains, the authors prepared stock saturated solutions of the oils with respect to water at room temperature, which for purposes of comparison may be considered to be between 20° and 25° C. The saturated values determined together with Clark's values are listed in Table IV.

Electric Hygrometer

The need of an apparatus for the determination of water in oil which would operate satisfactorily in the field is very apparent. With this in mind, the electric hygrometer described by Dunmore (*3*) was adapted to a procedure which removed the water from the oil at a temperature not greater than the normal operating temperature of the oil. In Figure 2, a schematic drawing of the apparatus is shown.

The oil was introduced by syringe through *E*, spraying into the evacuated chamber through the porous fritted disk, *A*. During this operation, the stopcock above *D* remained closed and the cold trap, *C*, was surrounded by a bath of dry ice. Stopcocks *F* and *G* were then closed and the contents of the trap and coil raised to some convenient temperature. The resistance of the film was measured either by a General Radio megohm bridge, as extended by Balsbaugh *et al.* (*1*), or a modified Jones and Joseph bridge (*10*) with a cathode ray tube as a null detector with three stages of amplification.

In Figure 3 is shown a plot of the logarithm of the resistance against the water content of the oils as determined by the combustion procedure. The drift in the resistance readings is illustrated by the insert curves. The change in resistance with time, although of a larger magnitude at the higher measured resistance, in terms of water content, is approximately the same as at the lower measured resistance. The resistance readings on the main curve were taken in each case after a 5-minute delay period.

Experiments are in progress on the simplification of the hygrometer construction and the selection of a more rugged alternating current resistance bridge of a suitable range. The method shows every promise of working out satisfactorily in the field.

Acknowledgment

The electrical bridges described in this paper were constructed in this laboratory under the direction of W. F. Davidson, director of research.

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A Laboratory Condenser

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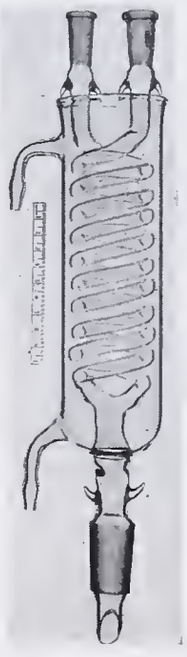
COOLING agents more effective than tap water are often needed in the laboratory condenser. In the ordinary straight condenser it is not convenient to use ice or other such refrigerant. The single-spiral condenser, suitable for downward distillation, does not usually have sufficient capacity for refluxing.

The double-spiral condenser shown was designed primarily to fill the need of an ice condenser which could be used for both downward distillation and refluxing. The coils are made from Pyrex glass tubing 9 mm. in inside diameter, and are carefully wound so that there are no traps in which liquid can accumulate.

It is apparent from the figure that tap water, ordinary ice, or solid carbon dioxide can be used as cooling agents.



This condenser has been particularly useful in this laboratory during the summer, when the tap water is often above 30° C. It has been very effective in the preparation of such products as acetaldehyde, hydrogen cyanide, and diazomethane. The ascending coil is an effective barrier against the evaporation of condensate.

The specimen shown was fabricated by the Scientific Glass Apparatus Company, Bloomfield, N. J.

Quantitative Determination of Dissolved Oxygen

Ascorbic Acid Oxidase Method

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DISSOLVED oxygen exerts the controlling role in many processes occurring in biological fluids. That this is true of milk has been amply demonstrated (*4, 8, 13, 14*). It is evident that to understand and control these oxidations a knowledge of the dissolved oxygen content is highly important. A simple rapid method for the determination of dissolved oxygen in milk is needed, especially at the present time, in view of the possibility of commercially deaerating pasteurized milk to preserve its vitamin C content and prevent the development of the oxidized flavor.

Methods for determining dissolved oxygen fall into several general classes.

The dissolved gases may be removed, the volume of mixed gases measured, and the mixture analyzed for carbon dioxide, oxygen, nitrogen, and water vapor. Complete removal of the dissolved gases is difficult and heating of the liquid in conjunction with reduced pressure is recommended (*10*). During the time required for removal at the temperatures recommended, some of the oxygen may react with other constituents of the biological fluid. This method is time-consuming and requires rather elaborate apparatus.

The amount of dissolved oxygen present in water low in organic matter, and from which certain interfering inorganic substances are absent, can be determined by adding to the water reagents that react quantitatively with the dissolved oxygen (*3, 17*). The well-known Winkler (*1, 17*) method is of this type. These methods are not applicable to biological fluids because the reagents are relatively strong, are not specific for oxygen, and react with organic material.

Methods have been described based on the reaction between dissolved oxygen and any one of a series of reducing agents to produce a colored compound, the amount of which is determined colorimetrically (*9, 11, 18*). Alkalies or acids are used to accelerate the reaction.

A quantitative method for dissolved oxygen, based on its reaction with reduced ascorbic acid added to the liquid, is reported in this paper. The reaction is completed in 5 to 15 minutes at 25° C., within the pH range 5.0 to 7.5.

In general, the literature indicates that one atom of oxygen reacts with one molecule of reduced ascorbic acid to form one molecule of dehydroascorbic acid. From the standpoint of a desirable analytical method the reaction in milk between dissolved oxygen and reduced ascorbic acid is too slow.

In the authors' early studies, after adding ascorbic acid, the reaction was catalyzed by adding 1 mg. per liter of copper as copper sulfate, after which the milk was kept cold for 2 days and the decrease in ascorbic acid was determined. Mixtures of deaerated and aerated milk were prepared and the composition of the mixtures as determined by oxygen content was found to agree within 1 to 2 per cent with the composition of the mixtures as prepared by weighing.

Next, work on the photosensitizing action of riboflavin on the oxidation of ascorbic acid by light in the presence of dissolved oxygen (*6*) led to the use of blue light as a catalyst. Completely filled test tubes containing a marble were rotated end over end before a strong blue light. The reaction between the dissolved oxygen and the reduced ascorbic acid was complete in less than 30 minutes. Although a large number of analyses were made using this catalyst, the absolute values obtained for dissolved oxygen raised doubts as to the quantitative nature of the reaction.

A study of the quantitative reaction between dissolved oxygen and reduced ascorbic acid (*5*) revealed that under various conditions 1.19 to 1.67 atoms of oxygen were involved with copper as the catalyst, 1.57 to 2.04 with light and riboflavin, 2.00 with alkali, and 1.00 with ascorbic acid oxidase (from cucumber juice or cabbage juice).

As a result of this study ascorbic acid oxidase was adopted as the catalyst and with a 10 to 1 concentrate of the enzyme the reaction was found to be complete in milk in 5 minutes at 25° C.

This general method for the determination of dissolved oxygen has been under investigation for 3 years and the method using cucumber juice as a catalyst has been in use for more than a year, during which time several thousand oxygen determinations have been made on milk, water, buffer solutions, bacteriological culture media, etc. With plenty of equipment conveniently arranged one person can make 30 determinations in a half day.

Reagents and Apparatus

PREPARATION OF DYE SOLUTION. Grind about 0.135 gram of 2,6-dichlorophenolindophenol in a mortar, add about 50 ml. of hot distilled water, grind further, and decant through a filter into a 1-liter volumetric flask. Add more hot water to the residue remaining in the mortar and again decant through the filter. Continue the process until practically all the blue color has passed through the filter, then adjust to room temperature, and make up to 1 liter with distilled water. The use of dye solution more than 2 weeks old is not advisable.

STANDARDIZATION OF DYE SOLUTION. Weigh accurately approximately 100 mg. of ascorbic acid, place in a 1-liter volumetric flask, and make up to volume with distilled water. Mix thoroughly and use at once for standardizing. Introduce 5 ml. of this standard ascorbic acid solution and 15 ml. of 0.1 *N* sulfuric acid into a 200-ml. beaker and titrate at once with the dye solution, using 15-ml. burets graduated to 0.05 ml. Titrate the solution to a light pink color which is permanent for 30 seconds. There is a tendency which must be guarded against to select darker and darker end points. Subtract from this titration the value obtained on a blank determination made by titrating a comparable acid water mixture to the same end point. The blank is usually about 0.3 ml. The dye factor in terms of milligrams of ascorbic acid per liter of milk when a 10-ml. aliquot of the milk is titrated is given by the following equation

$$\text{Factor} = \frac{\text{mg. of ascorbic acid weighed}}{2\ (\text{standardization titration} - \text{blank})}$$

The amount of original dye taken should be adjusted or the solution should be diluted to give a factor between 6.5 and 7.0.

SULFURIC ACID SOLUTION. Prepare a 10 *N* stock solution by diluting 285 ml. of concentrated sulfuric acid to 1 liter. For use, dilute 10 ml. of the stock solution to 1 liter and use approximately 25 ml. of this dilute solution for each determination.

ASCORBIC ACID OXIDASE. Freeze solid two to four medium to large green cucumbers, make 2 or 3 small holes about 0.6 cm. (0.25 inch) deep in the end of each, and suspend the cucumbers hole end downward in a piece of cheesecloth over a 1-liter beaker. At laboratory temperature the cucumbers will thaw and most of the juice (about 500 to 800 ml.) will drain out in 8 to 12 hours. A little squeezing of the bag will express an additional amount of juice. Most of the enzyme resides in the outer layer of the cucumber, and little is present in the seed portion. Therefore the yield of enzyme is not increased appreciably by excessive pressure or extraction of the pulp.

The juice may be concentrated by either freezing or pervaporation. In concentration by freezing about half of the water is frozen, the juice is expelled from the ice crystals, and about half of the water is then frozen in the expelled liquid. This process is continued until about 30 to 50 ml. of the juice concentrate remain. The concentrate is filtered and preserved by a drop or two of toluene or chloroform.

Concentration by pervaporation is more efficient. The expressed juice is placed in cellophane tubing about 1.8 cm. (0.75 inch) in diameter and the tube is looped over a glass rod. An electric fan is directed toward the loops. Evaporation to one

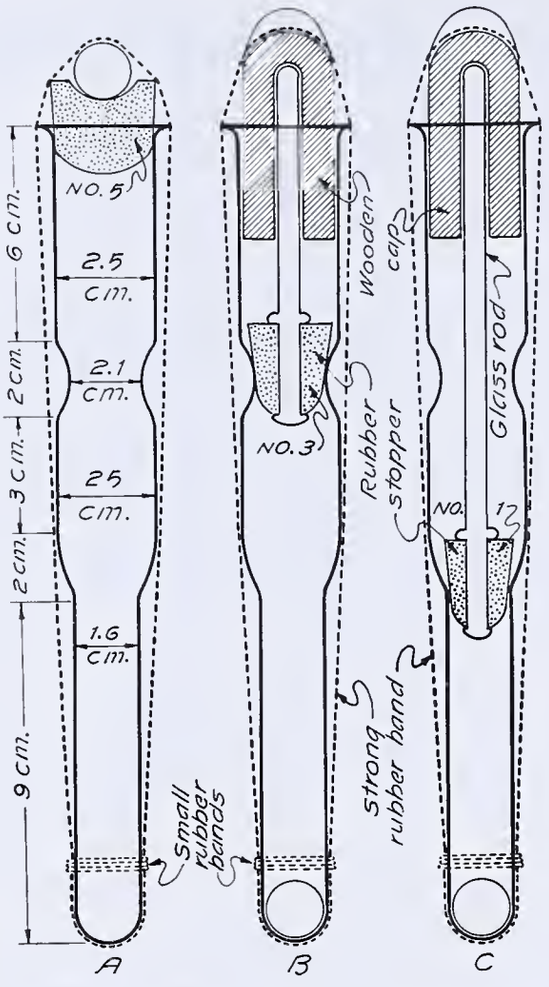

FIGURE 1. SPECIAL TUBE FOR DETERMINING DISSOLVED OXYGEN BY ASCORBIC ACID–ASCORBIC ACID OXIDASE METHOD

tenth to one fourteenth of the original volume will take about 8 hours. The temperature of the juice remains considerably below room temperature, owing to the cooling effect of evaporation. The concentrate is filtered and preserved by a drop or two of toluene or chloroform. For each determination 0.1 ml. of the concentrated juice is used.

The concentrate preserved in this way in the dark at 20° to 25° C. will maintain its activity for about 1 month; if kept cold it remains active for 2 months or more. The enzyme is destroyed very rapidly by shaking. Mercuric chloride, sodium chloride, and glycerol (1/2 volume), when used in bactericidal concentration, rapidly inactivate the enzyme. One-half saturation with ammonium sulfate can be used as a preservative, but it is not so satisfactory as toluene or chloroform. The enzyme can be precipitated with an equal volume of acetone and the dried precipitate possesses activity.

CONCENTRATED ASCORBIC ACID SOLUTION. Weigh 3.0 grams of *l*-ascorbic acid and 10 grams of sodium chloride and make up to 50-ml. volume with water. For each determination use 0.1 ml. of the solution.

SPECIAL TUBES FOR OXYGEN ANALYSIS. The tubes used for the oxygen determination are illustrated in Figure 1, which is largely self-explanatory.

The tube is so made that it can be closed at three levels, leaving no air trapped under the stopper after filling and after removing portions for titration. The filled tube with a No. 5 stopper in position *A* holds about 70 ml., with No. 3 stopper on a glass rod in position *B* about 45 ml., and with No. 1 stopper on a rod in position *C* about 20 ml. A large rubber band held over the small end of the tube by a small band holds the stoppers tightly in place. A round piece of wood partly drilled through is used to center and press downward on the glass rods. The upper end of this wooden cap is grooved, so that the band will not slip off.

In preparation for the taking of the sample the tube arranged as in *A* is used. A glass marble is used for mixing and is held in place in a depression cut on the top of the No. 5 stopper. The marble is mounted on the stopper of each tube, so that it will be in the hand while taking the sample and thus will not be forgotten after the sample is taken. The sample should not be taken with the marble in the tube, because air may be trapped between the marble and the bottom of the tube.

The authors have over 100 of these analysis tubes which were made by a glass blower. A large number of tubes was needed in studies of the oxygen content of milk which were carried out in commercial milk plants, and for studies on the rate of disappearance of oxygen from milk on holding.

TIME-SAVING DEVICES. The authors' work has required so many oxygen determinations that it has been found advisable to construct a number of devices to simplify and shorten the time required for the manipulation. These devices include a battery of 8 buret holders, beaker holders with pipet drainage support, oxygen analysis tube holder for shaking with spring clamps to hold in stoppers and tubes, a drainage rack, and various racks for holding the analysis tubes.

Method

COLLECTION OF SAMPLE. The special oxygen-analysis tube as illustrated in Figure 1, *A*, is used for collecting the sample.

Preferably the milk is made to flow from the vat by a glass or rubber-tube siphon or from a milk line by a petcock and rubber or glass tube. Hot milk can be siphoned or passed through a cold water–jacketed coil and thus discharged cold without exposure to air. The marble and stopper are removed from tube *A*, the end of the siphon is inserted to the bottom of the tube, the milk is made to flow steadily with undisturbed surface into the sampling tube, and one to two volumes are allowed to overflow. The siphon is then removed and the marble is placed gently on the surface of the milk in the analysis tube and released. If the marble is dropped into the milk a row of bubbles of air will follow in its wake. The rounded No. 5 stopper is then inserted. The rounding of the stopper aids in preventing the trapping of air and in expelling the surface milk. The rubber band placed over the stopper holds it under tension, so that if the milk is cooled further the stopper will follow the surface down and not permit air to be drawn in between the wall and the stopper. The sample is now ready for analysis.

Samples can be taken satisfactorily by means of a specially constructed pipet of about 250-ml. capacity. The pipet should be rather long and narrow, with a stem that is long enough to reach to the bottom of the special oxygen-analysis tubes. In sampling, the pipet is slowly drawn full of milk, the end is inserted to the bottom of the oxygen-analysis tube, and about two tube volumes of milk are allowed to overflow slowly; the pipet is then withdrawn, the marble added, and the stopper placed in the tube.

DETERMINATION OF REDUCED ASCORBIC ACID IN MILK. The analysis tube is inverted several times to mix the sample by causing the marble to fall from one end to the other.

Pipet 10 ml. of milk from the upper compartment of the tube into a 200-ml. beaker to which 25 ml. of dilute sulfuric acid have previously been added. Duplicate aliquots may be taken if desired. Titrate with the dye solution, using a 15-ml. buret calibrated in 0.05-ml. divisions. In titrating it will be found that after a small amount of dye is added the solution will assume a pink color. If this color fades on standing, more dye is added. Continue the addition of dye in portions until a light but definite pink color remains for 30 seconds. This is taken as the end point of the titration. Before calculating the original ascorbic acid in the milk a blank of about 0.4 ml. (*12*) should be subtracted.

In order to save the time which may be required for the pink color to fade it is convenient to have 6 or 8 burets and titrate 6 to 8 samples at one time. If one is interested only in the oxygen content, determination of the reduced ascorbic acid present in the original milk may be omitted.

ADDITIONAL ASCORBIC ACID ADDED. Fresh milk contains only about one third enough ascorbic acid to react with the dissolved oxygen present in ordinary milk; therefore ascorbic acid must be added in amount to be amply sufficient to react with all the oxygen which may be dissolved in the milk.

Add 0.1 ml. of the concentrated ascorbic acid solution, using a 1-ml. buret pipet graduated to 0.01 ml. To the end of this pipet a small glass tube 25 cm. long and 3 mm. or less in outer diameter is attached by means of a short piece of rubber tubing 3 mm. in outer diameter and 1 mm. in internal diameter. The flow is controlled by a spring pinchclamp. Insert the glass tube to the bottom of the analysis tube and add 0.1 ml., making this addition to the bottom of the tube, so that none of the solution will be expelled with the overflow when the stopper is inserted later. If this buret is held in the hands ascorbic acid can be added rapidly to a number of tubes in succession.

The tube is stoppered according to the arrangement in Figure 1, *B*. The volumes have been so worked out that the surface milk is displaced in seating the stopper. Discard this excess milk and invert the tube continuously for 1.5 minutes to ensure complete mixing of the added ascorbic acid with the milk. Then remove the stopper and titrate an accurately measured 5-ml. portion, and a duplicate if desired, for ascorbic acid according to the procedure described above. The buret reading should be between 13 and 15 ml.

Addition of Ascorbic Acid Oxidase. After removal of the sample to determine the original plus added ascorbic acid, add to the bottom of the analysis tube 0.1 ml. of the concentrated cucumber ascorbic acid oxidase, using a 1-ml. buret graduated to 0.01 ml., similar to that used for adding the ascorbic acid. The same buret should not be used, however, because great care should be taken to prevent contamination of the other burets and equipment with the ascorbic acid oxidase concentrate. Close the tube with the stopper according to the arrangement in Figure 1, *C*. Mix the contents of the tube by repeated inversion for 1 minute, and allow the tube to stand for 15 minutes, during which time the enzyme causes all the dissolved oxygen to react with the reduced ascorbic acid. Pipet 5 ml. and a duplicate, if desired, from the lowest compartment of the tube directly into 24 to 26 ml. of 0.1 *N* sulfuric acid previously placed in the beaker in preparation for the titration. The acid inactivates the enzyme and prevents further fast reaction between the air to which the aliquot is now exposed and the remaining ascorbic acid.

It is very necessary to make this titration immediately after pipetting out the sample. Although tests indicate that the enzyme is inactivated, nevertheless the rate of ascorbic acid oxidation is fairly rapid. Enough ascorbic acid should be present at the start so that this final titration does not fall below 2 ml. The 5-ml. pipet used in removing samples for this final titration should be reserved for the measurements involving this step only, because of the danger of producing errors in other titrations as a result of contamination with ascorbic acid oxidase.

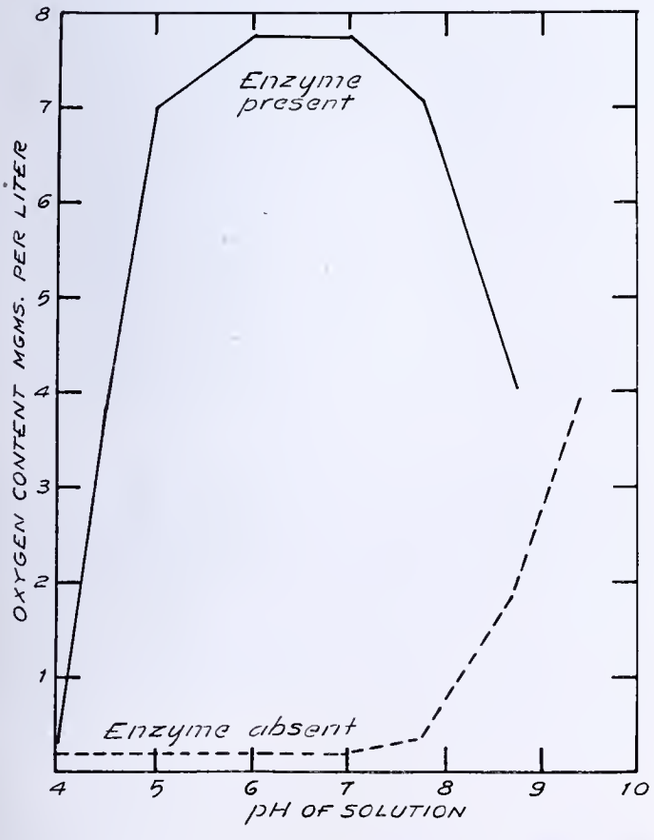

Figure 2. Activity-pH Range of Cucumber Juice Ascorbic Acid Oxidase

Calculation. The dye is standardized against pure ascorbic acid, so that when 10 ml. of milk are titrated, the milliliters of dye used less the blank times the factor gives the milligrams per liter of ascorbic acid in the milk.

The oxygen content in milligrams per liter of milk is obtained by multiplying the difference in dye titration before and after adding the ascorbic acid oxidase by twice the dye factor divided by 11. The dye factor is multiplied by 2 because only 5 ml. of milk were titrated and divided by 11 because the molecular weight of ascorbic acid is eleven times the atomic weight of oxygen.

From the oxygen content in milligrams per liter as calculated above subtract 0.45 mg. per liter for the blank resulting from the small contaminations with oxygen which occur during manipulation.

Evidence for Reliability of the Method

Titration Blank on Enzyme Added. The possibility of the enzyme's possessing dye-reacting properties, the ability of the sulfuric acid to stop enzyme action, and the duplicability of titrations were indicated by results obtained in a series of experiments carried out in conjunction with some routine oxygen determinations.

Two 5-ml. aliquots were removed from the middle compartment of each tube after incorporating the ascorbic acid, and were added directly to 25-ml. portions of the 0.1 *N* sulfuric acid in the 200-ml. beakers. To one beaker was then added 0.1 ml. of the ascorbic acid oxidase concentrate. Both beakers were titrated about 2 minutes later.

In all, 80 pairs were titrated and in 42 the difference was 0.1 ml. of dye or less. The greatest difference was 0.55 ml., the next greatest 0.45 ml. The average deviation was 0.14 ml. with signs disregarded and 0.04 ml. with signs considered. Since 0.1 ml. of the enzyme extract was titrated in this case, whereas only about one fourth of this amount would be present in the 5-ml. aliquot removed in the oxygen determination, the amount of dye reacting with the constituents of the enzyme extract is negligible. This was confirmed by titrating 5 ml. of the enzyme extract directly. These results also indicate the agreement expected in duplicate titrations.

pH Range of Ascorbic Acid Oxidase Activity. The pH range over which solutions could be analyzed for oxygen without pH adjustment was determined by adding limited amounts of ascorbic acid oxidase to aerated buffer solutions. An average composite curve was constructed and is presented in Figure 2. Blank determinations for the decrease in ascorbic acid in the buffer solutions in the absence of added oxidase were also made. The kind and dilution of the buffer influenced the blank in the range from pH 4 to 7. At pH 8 the enzyme was inactivated, but the alkali in the absence of enzyme caused a decrease in the amount of ascorbic acid. The enzyme was almost completely inactive at pH 4. The enzyme in the pH range of 5 to 7.5 has sufficient activity to complete the reaction in the allotted 15 minutes.

The optimum pH range for the action of ascorbic acid oxidase from various sources is reported as follows: Tauber, Kleiner, and Mishkind (*16*) 5.56 to 5.93 and 5.38 to 5.57 for Hubbard squash; Srinivasan (*15*) 4.6 to 5.6 and 5.3 to 5.6 for *Moringa pterygosperina;* Ito (*7*) 6.0 for cucumber juice; and Ebihara (*2*) 6.0 to 6.5 for cucumber juice. The values reported for cucumber juice agree well with those in Figure 2. Since several buffers as well as milk and whey were used, the authors believe Figure 2 indicates the activity range of ascorbic acid oxidase from cucumber juice.

Effect of Amount of Enzyme Added. Numerous experiments indicate that an excess of enzyme exerts no influence on the oxygen determination. Only one such experiment is cited here as an example.

Table I. Comparison of Oxygen Determinations on Water

Oxygen Content		Oxygen Content	
Winkler	Ascorbic acid oxidase	Winkler	Ascorbic acid oxidase
Mg./l.	*Mg./l.*	*Mg./l.*	*Mg./l.*
0.00	0.01	5.2	5.2
0.00	0.07	5.8	6.0
0.00	0.08	6.1	6.0
0.02	0.00	8.2	8.0
1.6	1.6	9.8	9.7
2.0	1.9	10.1	10.3
2.9	2.7	10.8	10.6
4.1	4.1	10.9	10.7

Enzyme in the following amounts was added to a series of tubes of milk: 0.05, 0.10, 0.20, 0.30, 0.50, and 1.00 ml. The amounts of oxygen obtained were 8.24, 8.40, 8.18, 8.32, 8.41, and 8.45 mg. per liter, after corrections were made for the oxygen content of the enzyme concentrate.

Blank on Boiled Oxygen-Free Water. A 5-liter short-necked round-bottomed Pyrex flask, three fourths full of water, was made to boil vigorously. After about one fifth and in some cases as much as one third of the water had been evaporated, samples were removed for oxygen determination by siphoning without interrupting the boiling. The siphon tube reached to the bottom of the boiling flask. The outer end of the siphon tube was made of coiled aluminum tubing surrounded by a cooling bath; thus cooled oxygen-free water was delivered at the lower end of the siphon. Three volumes of water were allowed to flow through the siphon and overflow the oxygen-analysis tube before the sample was taken for analysis. At various times 48 such samples were taken. The results obtained indicated a blank of 0.44 mg. per liter for oxygen-free water. Similar samples analyzed by the Winkler method gave a blank of 0.10 mg. per liter, including the blank on the reagents.

Blank on Oxygen-Free Milk. Milk was deaerated by a process which involved subjecting milk to a pressure sufficiently low to make the milk boil and thus convert some of its water into vapor, which was used as the agent to sweep out the dissolved oxygen. For these tests the pressure utilized was sufficiently low to decrease the temperature of the milk about 20° C. Determinations were made on 123 samples prepared in this way.

The average blank was 0.45 mg. of oxygen per liter of milk. The mean deviation from the average blank was 0.14, the range from 0.05 to 0.80, while 64 samples were found to deviate by 0.1 mg. or less from the average blank. These deviations are about those that would be encountered in duplicate titrations. The blank on milk agrees well with the average value of 0.44 obtained with water.

A few blank determinations should be run on each lot of enzyme, because occasionally for some unknown reason the blanks are higher.

Comparison between Winkler and Oxidase Method. A series of samples of water was analyzed for oxygen by both the Winkler and the ascorbic acid oxidase methods. The results obtained are presented in Table I. The difference between the results by the two methods is not significant. A blank of 0.10 mg. was subtracted from the results by the Winkler method and of 0.44 from the results by the ascorbic acid oxidase method.

Example. As an example of the variations in results which might be expected when the determinations are made by one conversant with the method and particularly by one trained in determining the end point of the dye titration, Table II is presented. This gives results obtained with samples taken to test a particular run made on a commercial milk deaerator. Conversion factor for dye used was 1.20. Blank on method and reagents was 0.45 mg. per liter of oxygen.

Milk from the cans received from the dairy barn was dumped directly into the raw milk vat, the milk was gently agitated, and the samples were removed. Most raw milk at the time it enters the pasteurization process is much higher in oxygen than the raw milk of Table II because of passage through weigh tanks, pumps, storage vats, etc. The milk from the raw-milk storage vat was sucked into a milk deaerator and deaerated (*13*). The milk was pumped out of the deaerator and samples were taken from the line leading from the pump. The analyses of these samples represent the extent of deaeration.

By means of this pump the milk was forced through a plate heater, by which the milk was heated to 71.6° C. (161° F.) through a holding pipe (16 seconds), through a plate cooler, and finally into a surge tank, the surface of which was protected from air by a float which rested on the surface of the milk and rose and fell with the variations in the amount of milk in the surge tank. The milk passed to the filler bowl tank on the bottler by gravity. The third set of samples was taken from this line and represents the amount of air incorporated in the series of steps following deaeration.

The final set of samples was taken from the filled quart bottles and represents the amount of air incorporated in filling the bottles, using conventional valves but with a protecting float on the surface of the milk in the filler bowl.

Errors in the end point of the dye titration can be reduced by duplicate determinations on each tube. The results in Table II represent only single titrations on the various portions in each tube. Table II indicates the variations which might be expected in applying this method to samples taken at intervals during the run of a milk deaerator.

Table II. Oxygen Content of Milk

(Samples taken at various time intervals during operation of a continuous commercial market milk deaerator)

	Dye Titration			Oxygen Content	
Stage of Sampling	Start	After enzyme	Difference	Difference, ml. × 1.2	After blank, 0.45
	Ml.	*Ml.*	*Ml.*	*Mg./l.*	*Mg./l.*
Raw milk in vat	11.25	6.45	4.80	5.76	5.31
	11.15	6.25	4.90	5.88	5.43
	12.05	7.15	4.90	5.88	5.43
	11.40	6.55	4.85	5.82	5.37
	11.75	7.80	3.95	(4.74)	..
	12.00	6.95	5.05	6.06	5.61
				Mean	5.43
After deaeration, sample following deaerator pump	13.90	13.35	0.55	0.66	0.21
	11.60	11.00	0.60	0.72	0.27
	11.60	10.90	0.70	0.84	0.39
	13.25	12.85	0.40	0.48	0.03
	12.55	12.10	0.45	0.54	0.09
	12.10	11.65	0.45	0.54	0.09
	13.00	12.60	0.40	0.48	0.03
	13.00	12.55	0.45	0.54	0.09
				Mean	0.15
After pasteurization and cooling, pipe line from surge tank to filler bowl	11.45	10.90	0.55	0.66	0.21
	12.45	11.85	0.60	0.72	0.27
	11.55	11.00	0.55	0.66	0.21
	12.00	11.45	0.55	0.66	0.21
	14.40	13.90	0.50	0.60	0.15
	11.95	11.35	0.60	0.72	0.27
	13.30	12.65	0.65	0.78	0.33
	12.05	11.55	0.50	0.60	0.15
				Mean	0.23
Milk from bottles	12.35	10.90	1.45	1.74	1.29
	11.80	10.40	1.40	1.68	1.23
	11.45	10.05	1.40	1.68	1.23
	12.20	10.70	1.50	1.80	1.35
	10.85	9.00	1.85	2.22	1.77
	13.10	11.45	1.65	1.98	1.53
	11.50	10.15	1.35	1.62	1.17
	11.90	10.60	1.30	1.56	1.11
				Mean	1.33

Summary

The dissolved oxygen content of several fluids containing organic matter can be determined quantitatively by adding reduced ascorbic acid followed by ascorbic acid oxidase. The decrease in ascorbic acid as determined by titration indicates the dissolved oxygen content.

Special oxygen-analysis tubes were designed to protect the liquid from air and avoid transference of the liquid during the determination.

The method shows good agreement on water when compared with the Winkler method.

The probable error is 0.1 to 0.2 part per million.

One analyst can easily make 30 determinations in a half day if sufficient conveniently arranged equipment is available.

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Spectrochemical Analysis of Trace Elements in Fertilizers

ZINC

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FROM year to year an increasing number of plant nutrition experiments have shown that many serious deficiency diseases are due solely to an inadequate supply in the soil of minor plant food elements that are essential for normal plant growth. In a list of such elements—the so-called secondary plant nutrients—zinc has been accorded a prominent place. Thus, the white bud of corn, pecan rosette, bronzing of the tung-tree leaf, citrus little leaf, and leaf wilt of tobacco have all been ascribed to zinc deficiencies (*3*). The toxic effect of large quantities of the element, on the other hand, is also well recognized. Consequently a knowledge of the concentration of zinc in fertilizer materials and mixed fertilizers, added directly to the soil to enrich the plant food, becomes desirable.

In previous studies (*5*, *6*) of the concentrations of certain secondary plant food elements in mixed fertilizers by spectrochemical analysis, the determination of zinc proved to be somewhat difficult. Zinc can be readily detected by the pairs of arc lines 3302.6–3303.0 A. and 3345.0–3345.5 Å. and the three persistent lines 4680.1 Å., 4722.2 Å., and 4810.5 Å. The first of these, the 3302.6–3303.0 Å. pair, is, in most spectra, masked by traces of the sodium doublet 3302.3–3303.0 Å. and unsuitable for quantitative determination. Of the other lines the pair 3345.0–3345.5 and the 4810.5 Å. line have in particular been adopted by others for the quantitative determination of zinc. However, in the complex spectrum of a mixed fertilizer, containing only a small per cent of zinc, these lines are not sensitive enough in the direct current arc source to overcome background sufficiently to permit intensity measurements with any degree of accuracy. Study has shown that the high-voltage alternating current arc source is a more sensitive method for zinc, and, furthermore, has the advantage of producing spectra comparatively free of background. Unfortunately, however, it has also been shown in this laboratory (*6*) that this method of excitation cannot be used for the analysis of fertilizer samples, but is restricted to solutions or very homogeneous solids.

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Consequently, it has been necessary to abandon an attempt to determine zinc simultaneously with the other important secondary nutrients, boron, manganese, and copper in the fertilizer samples, and an effort has been made to find or devise a spectrochemical method for the quantitative determination of zinc that could be applied to a mixed fertilizer sample. The purpose of this paper is to describe: (1) the modifications in the spectrochemical procedure that have been adopted for the quantitative determination of zinc in mixed fertilizers and (2) the application of this method to the analysis of 44 representative mixed fertilizer samples whose complete composition has previously been determined by chemical methods in this laboratory (*2*).

Apparatus and General Procedure

The apparatus used, a large Hilger Littrow quartz spectrograph, a water-cooled arc stand operated by means of a direct current at a 220-line voltage, and a Bausch & Lomb nonrecording densitometer, have been described (*5*, *6*). The only modification in the apparatus has been the addition of two Nichrome wire resistance boards, inserted in the electrical circuit in parallel with the water-cooled rheostat, to permit the use of higher currents. With this arrangement any desired current from 5.0 to 25.0 amperes can be conveniently obtained.

As in the analyses for boron, manganese, and copper the direct current arc method of excitation using a cupped graphite anode and a pointed graphite cathode was selected. The well-known logarithmic step-sector method of Scheibe in conjunction with the internal standard method of Gerlach was retained and the samples were again analyzed without any preliminary chemical treatment.

Modified Experimental Procedure for Zinc

A method satisfactory for an accurate spectrochemical determination of zinc in a mixed fertilizer would include the measurement of a zinc line of greater sensitivity than those ordinarily used, and/or photographing under conditions where the background produced is considerably less. Rogers (*8*) investigated the use of the zinc line at 2138.6 Å. in order to avoid preliminary chemical treatment in the analysis of

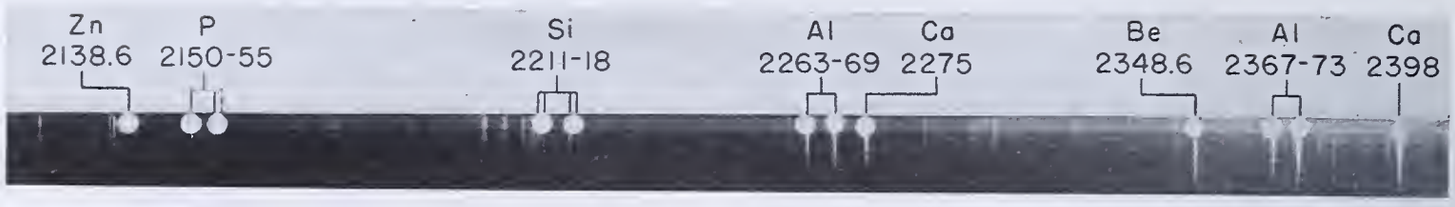

FIGURE 1. SPECTROGRAM OF MIXED FERTILIZER SAMPLE FOR QUANTITATIVE DETERMINATION OF ZINC

various plant materials. Using photographic plates treated with a special ultraviolet sensitizing material, this line can be photographed at appreciably higher intensities than any of the zinc lines in the near ultraviolet, and, except with very long exposures, the background in this region is practically negligible.

Several investigators have shown that when a rapidly volatilizing material is consumed in a direct current arc more intense lines can be obtained by the use of higher currents. Consequently, the effect of current on the rapidly volatilizing zinc was studied. These studies showed that for the volatilization of a given amount of sample the sensitivity of the zinc line at 2138.6 Å. does not change appreciably with increasing current. However, the higher current permits the complete volatilization of a larger sample without increasing the exposure time and when a larger sample and higher current are used, the arc strikes more directly to the sample, completely volatilizing it while consuming relatively less of the graphite holder. This results in effectively increasing the blackness of the zinc line over its background. For this reason throughout the zinc analyses a current of 20 amperes and a sample of 20 mg. were adopted.

When duplicate analyses of the fertilizer samples for zinc were first attempted under these conditions, inconsistently high results were encountered at irregular intervals. Further investigation showed that certain samples, in particular when the silicon content was high, did not completely volatilize before the graphite cup was consumed. Consequently, last traces of the sample, presumably containing some of the slowly volatilizing beryllium, added to the fertilizer sample as the constant internal standard, were lost. This would mean that the intensity of the beryllium line would be weakened. Consequently, the ratio of the intensity of the zinc line to the intensity of the beryllium line and the resulting value for the concentration of the zinc would be too high.

This difficulty is most readily overcome by increasing the depth of the graphite cup to assure complete volatilization of the sample. Mannkopff and Peters (4) have studied the effect of cup depth best suited for various substances when they have different melting points, and Pierce, Torres, and Marshall (7) reached the conclusion that with readily volatile elements higher intensity may be obtained if the sample is placed in a deep cup. This conclusion was confirmed in the present investigations with zinc. When the cup depth was increased from 6 to 10 mm., not only was complete volatilization of the sample assured, but the sensitivity of the zinc line was increased. If the sensitivity of a spectrum line can be sufficiently increased, it can be photographed, by use of shorter exposure time, entirely free from background. In the present case, where a stepped sector is used during each exposure, this means that if the sensitivity can be sufficiently increased, the line can be measured at a step where it is free from background. When the fertilizer samples were volatilized from deep cups, the zinc line was increased in sensitivity, so that measurements entirely free from background could be made even in those cases where the zinc concentration in the fertilizer was extremely small.

The conditions finally adopted for the analysis of zinc were: a direct current arc maintained by a 220-line voltage at 20 amperes current, an electrode separation of 4 mm., and a slit 7 mm. high and 0.06 mm. wide. The cathode is a pointed graphite rod 0.47 cm. (0.19 inch) in diameter and the anode a graphite rod 1.25 cm. (0.25 inch) in diameter into which a flat-bottomed cup 10 mm. deep, 4.3 mm. in diameter, and with a wall 0.5 mm. thick, has been cut. A 20-mg. sample of the mixed fertilizer is placed in this cup, the spectrograph is adjusted to photograph the region 2000 to 2450 Å., and the sample is arced for 2.5 minutes. The intensities of the zinc line at 2138.6 Å. and the beryllium line at 2348.6 Å. are measured and the ratio of these intensities is used to determine the zinc concentration from the working curve. Eastman 1-0 spectroscopic ultraviolet sensitive plates were selected as the fastest obtainable. The plates were constantly brushed during development in Eastman developer D-76c for 5.5 minutes at 18° C. Figure 1 shows a section of the spectrum of a fertilizer sample when photographed under these conditions.

TABLE I. COMPARISON OF SPECTROCHEMICAL ANALYSIS FOR ZINC WITH CHEMICAL VALUES

Fertilizer Grade[a]	Source	Year Sampled	Spectrochemical[b]	Chemical[c]	Ratio Chemical/ Spectrochemical
			Av. %	%	
1*-9-4	N. Y.	1929	0.0415	0.032	0.77
2*-8-10	N. Y.	1929	0.0144	0.012	0.83
2-12-6	Ohio	1929	0.0084	0.033	3.81
7-5-4	Md.	1926	0.0092	0.0080	0.87
4*-8-4	Va.	1930	0.092	0.0080	0.09
3-8-3	Va.	1930	0.012	0.0080	0.67
4*-8-4	N. C.	1925	0.015	0.0080	0.53
4*-8-4	Va.	1926	0.0261	0.060	2.30
3-8-5	Md.	1930	0.036	0.0080	0.22
4*-12-4	Va.	1930	0.0185	0.048	2.59
5*-8-5	Penna.	1929	0.0100	0.032	3.20
5*-8-7	Va.	1930	0.062	0.048	0.77
7*-6-5	Va.	1930	0.059	0.004	0.07
2-9-5	Md.	1935	0.00495	0.0080	1.62
2-12-2	Ind.	1935	0.014	0.012	0.86
2-12-4	Md.	1935	0.014	0.0080	0.57
3*-9-3	Ga.	1935	0.00385	0.0080	2.08
4*-8-4	Ga.	1935	0.0255	0.032	1.25
3-8-6	N. C.	1935	0.047	0.000	–
4*-8-6	S. C.	1935	0.00375	0.0016	0.43
4-8-4	La.	1935	0.0255	0.000	–
4-8-5	Md.	1935	0.031	0.016	0.52
4-8-7	N. Y.	1935	0.065	0.016	0.25
4-10-7	Va.	1935	0.0088	0.008	0.91
4-12-4	Ark.	1935	0.014	0.014	1.00
6-6-5	Va.	1935	0.018	0.024	1.33
6*-8-6	Mass.	1935	0.0099	0.008	0.81
3-21-6	Ohio	1929	0.108	0.064	0.59
4-16-4	N. Y.	1935	0.0445	0.020	0.45
4*-16-10	Penna.	1930	0.045	0.016	0.36
4-24-12	Ind.	1934	0.031	0.032	1.03
8-12-20	Maine	1935	0.253	0.012	0.05
8*-16-8	Ga.	1927	0.033	0.144	4.36
8-16-12	Minn.	1933	0.049	0.045	0.92
8*-16-16	Mass.	1935	0.080	0.040	0.50
9*-27-9	Va.	1928	0.0233	0.072	3.09
12-24-12	Germany	1930	0.0125	0.008	0.64
10-30-10	N. J.	1929	0.0185	0.004	0.22
17-34-17	Ala.	1932	0.0275	0.028	1.02
0-10-4	Tenn.	1935	0.0241	0.036	1.49
0-10-10	N. Y.	1929	0.034	0.004	0.12
0-12-5	Md.	1935	0.030	0.012	0.40
0-12-12	Ind.	1934	0.030	0.008	0.27
0-14-6	Ohio	1926	0.036	0.008	0.22

[a] Grade formulas stand for percentages of N, P_2O_5, and K_2O in this order except that numbers indicated by asterisk stand for percentages of NH_3.
[b] Values expressed as element in accordance with recommendation adopted by the Association of Official Agricultural Chemists.
[c] Chemical results from Lundstrum and Mehring are converted to per cent of element (Zn) to facilitate comparisons.

Table II. Precision of Spectrochemical Analysis of Zinc

Trial	Concentration	Deviation from Mean
	%	%
1	0.015	0.0006
2	0.013	0.0014
3	0.015	0.0006
4	0.017	0.0026
5	0.014	0.0004
6	0.012	0.0024
7	0.016	0.0016
8	0.013	0.0014
	Av. 0.0144	0.00138

Table III. Accuracy of Spectrochemical Analysis of Zinc

Fertilizer Grade[a]	Source	Year Sampled	Zn Added	Original Spectrochemical Analyses	Subsequent Spectrochemical Analyses
			%	*Av.* %	%
1*–9–4	N. Y.	1929	0.032	0.0415	0.0758
4*–8–4	Va.	1926	0.032	0.0265	0.0496
4*–8–4	Ga.	1935	0.016	0.0255	0.0386
6*–8–6	Mass.	1935	0.016	0.0099	0.0268
12–24–12	Germany	1930	0.048	0.0125	0.0569
0–14–6	Ohio	1926	0.048	0.0360	0.0819

[a] Grade formulas stand for percentages of N, P_2O_5, and K_2O in this order, except that numbers indicated by asterisk stand for percentages of NH_3.

A series of standards prepared by duplicating as closely as possible the average composition of the 44 mixed fertilizers as determined chemically was available from previous analyses of the fertilizer samples. As these standards had been found to be entirely satisfactory for the analyses of boron, manganese, and copper and as they contained zinc in concentration from 0.0008 to 0.4 per cent and 0.05 per cent beryllium as an internal standard, they were used to prepare a working curve for the zinc analyses, although the choice of an element as an internal standard which volatilizes at a rate closer to that of the rapidly volatilizing zinc than does the slow-volatilizing beryllium would have been more advantageous. Figure 2 shows the straight-line working curve obtained by plotting the intensity ratios against the known zinc concentrations, the circular and triangular symbols representing two different sets of standards photographed on separate plates.

Discussion of Results

The results obtained from duplicate analyses of the fertilizer samples are listed in Table I. The average deviation from the mean of the duplicate determinations of the 44 samples is ±4.88 per cent. The deviations range from a minimum of 0.0 per cent to a maximum of ±12.0 per cent and with but three exceptions are within ±10.0 per cent.

As with the boron, manganese, and copper determinations, precision and accuracy tests have been made during the zinc analyses. Table II lists the results obtained from repeated analyses of the same fertilizer sample from time to time during the course of the zinc analyses. The arithmetical mean and the average deviation from the mean are given at the bottom of the second and third columns, respectively. From the data in this table the probable error of the mean, 0.6745 $\sqrt{\frac{\Sigma(d^2)}{\eta(\eta-1)}}$, is found to be 0.000402. Table III lists the results obtained from repeated analyses of six fertilizer samples, selected at random, after known amounts of zinc had been added to each. These results, while insufficient for any statistical analysis, show that the accuracy is of the same order as the precision.

Thus, the method devised for the analysis of zinc is shown to be capable of a precision and accuracy equal to those obtained in the analysis of boron, manganese, and copper. The use of plates which are treated with a special sensitizing material, which must subsequently be removed, for accurate quantitative work is thereby justified. With measurements entirely free from background a somewhat higher precision might have been expected (*5*). However, the use of faster plates which never appear to be as clear and as free from chemical fog as the slower process plates used for the boron, manganese, and copper determinations, as well as possible slight variations in the sensitizing material, may counterbalance this advantage.

Table I also gives a comparison between the average spectrochemical values and the chemical values obtained by Lundstrom and Mehring (*2*). In general, the chemical values tend to be somewhat lower than the spectrochemical. This same tendency was found by Rogers and Gall in a comparison of spectrochemical and chemical methods for the determination of zinc in plant ash (*9*). The mean of their spectrochemical values was usually appreciably higher than the corresponding average of the chemical values. In the results compared in Table I, the difference between the values obtained by the two methods is usually within a twofold factor—i. e., the ratio of chemical to spectrochemical is within a variation of 0.50 to 2.00. When the average of the spectrochemical values is particularly high, approaching or exceeding 0.1 per cent (the chemical values with but a single exception never exceeding 0.09 per cent), and when the chemical value is reported as 0.000 per cent, the agreement becomes particularly poor.

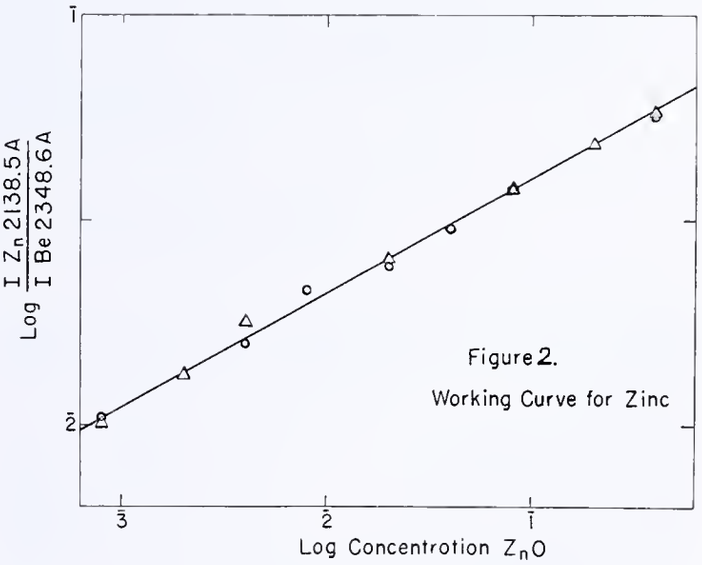

Figure 2. Working Curve for Zinc

From the working curve, Figure 2, concentrations of zinc from 0.0008 per cent (8 parts per million) to 0.4 per cent (4000 parts per million) can be determined. These values represent only the limits established for the analysis of the 44 fertilizer samples, where the concentrations always fall well within these limits. The upper limit of the method has not been investigated but, as pointed out by Rogers (*8*), is probably not much greater than 1 per cent where reversal of the 2138.6 Å. zinc line would be encountered. However, when concentrations of this magnitude are reached, the objection to the lines in the near ultraviolet would no longer be valid.

Tests have been made which show that the lower limit of the method cannot be extended below about 0.0002 per cent (2 parts per million).

Possible interference of an iron line at 2138.6 Å. has been suggested by Rogers (*8*). Harrison (*1*) assigned this line a relative intensity in the arc source of only 8, as compared to the zinc line 800; hence interference would not be expected until the iron concentration became relatively high. However, tests were made concerning the possible interference of this line and a copper line at 2138.5 Å. to which Harrison assigns an intensity value of 25. These tests show that, under the conditions of the zinc analyses, 3.0 per cent iron and 1.0 per cent copper, values in excess of those ever found in the fertilizer samples by previous chemical and spectrochemical analyses, give no measurable densities of the lines at 2138.6 and 2138.5 Å., respectively.

Summary

A method has been described for the spectrochemical determination of zinc in mixed fertilizers and the method applied to the analysis of 44 representative mixed fertilizers of known chemical composition. The method is capable of determining zinc within the limits of 0.0002 per cent (2 parts per million) to approximately 1 per cent (1 part per hundred) with an accuracy within ±5 per cent, thus equaling methods previously described for the analysis of boron, manganese, and copper. As the method involves the use of plates which have been treated with a special sensitized coating, the accuracy obtained confirms the fact that these plates are sufficiently consistent to permit quantitative determinations without appreciable sacrifice of accuracy. Comparison of the values obtained by the spectrochemical method with those obtained by chemical analysis show that in general the chemical values are lower than the spectrochemical values, a discrepancy previously noted elsewhere in similar comparisons.

The method described augments the number of essential trace elements whose concentrations could be guaranteed by supplemental spectrochemical analysis following the usual chemical determinations of the primary plant nutrients. The determinations when the concentration of zinc exceeds 1 per cent can be readily achieved by use of the zinc lines at 3345.0 or 4810.5 Å.

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Extraction of Carotene from Plant Material

A Rapid Quantitative Method

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MOST methods for the determination of carotene in plant material require extraction of the sample for a considerable period of time, and some materials must be ground in a mortar with sand up to 20 minutes prior to extraction.

Coleman and Christie (*1*) reported a rapid method for the extraction of carotenoids from flour using a high-speed stirrer. Davis (*2*) used a food blender in cutting up foods for sampling, and research workers of the Agricultural Chemistry Department of Purdue University (*4*) used this blender for extraction purposes. It consists of a quart heavy glass container of cloverleaf design in cross section, at the bottom of which is an assembly consisting of knives turned by a motor at a speed of about 10,000 r. p. m. Thus the material is subjected not only to considerable maceration by the knives but also to agitation.

This paper presents a rapid quantitative method for the extraction of carotene from certain plant materials, using the food blender (manufactured by the Waring Corporation, New York, N. Y.) in conjunction with a foaming solvent.

Experimental

Several solvents were tried in extracting the carotene from plant material but because of the high speed of the blades and an attempt to use as small an amount of solvent as possible, there was considerable splashing and jumping of the solvent. Quantitative results were therefore not attainable unless loss of the solvent from the top of the container could be prevented. By experimentation it was found that a certain mixture of 95 per cent ethyl alcohol and petroleum ether (Skelly-solve B used throughout this work) produced a foam which prevented this splashing and jumping of the solvent. In order to study the limitations of concentration of the two constituents of the foaming solvent the following experiment was performed.

A total of 200 ml. of varying concentrations of 95 per cent ethyl alcohol and petroleum ether were made up in 250-ml. Erlenmeyer flasks, which were placed in a water bath at 27° C. The various mixtures were then successively placed in the container of the blender and the time in seconds was noted for the last bubble of the foam to disappear after the motor had been turned off. Both constituents had an initial 3-second period for the last bubble to disappear, which in reality was the time required for the knives to stop after the motor had been turned off. The results are shown in Figure 1.

Fresh green samples were cut up with scissors sufficiently fine for accurate sampling. Silage samples were ground through a food chopper preparatory to sampling. One to 4 grams of the prepared sample were weighed out and transferred to the container of the blender.

The foaming mixture used in this study was approximately 57 per cent of 95 per cent ethyl alcohol by volume. It consisted of 100 ml. of 95 per cent ethyl alcohol and 75 ml. of petroleum ether which was added to the blender containing the sample. The blender was then run for 5 minutes, which was sufficient time for macerating the sample and extracting the carotene. Sometimes it was necessary to add more alcohol to the mixture to induce foaming. The lack of foaming is due to the water contained in the sample.

The residue was permitted to settle, and the supernatant liquid was transferred to a separatory funnel with the aid of a glass rod and a small funnel. Sufficient water was added to make the alcohol concentration equal to about 80 per cent, after which the hypophase was separated from the epiphase. The residue was then washed with 30 ml. of petroleum ether, which in turn was used to extract the alcohol layer. The residue and alcohol layer were again twice treated with 30 ml. of petroleum ether. The combined petroleum ether extracts were washed with tap water six or seven times to remove all the alcohol. The extract was concentrated under vacuum in an Erlenmeyer flask to about 25 ml., the process being hastened by running hot water over the flask. The carotene was then separated from the other pigments by passing the extract through a column of dicalcium phosphate (*3*). The filtrate was made up to volume and the carotene concentration evaluated with a photoelectric colorimeter.

With dried samples some difficulty was encountered in obtaining quantitative extraction. Commercial dehydrated alfalfa-leaf meals could be extracted quantitatively without any difficulty. However, the carotene was not extracted quantitatively from hay samples that were put through the 1-mm. screen of a Wiley mill. This difficulty was overcome by placing the weighed sample (2 grams) on a filter paper in a funnel and washing with 25 ml. of hot tap water (60 to 70° C.). The excess water was drawn off by light application of vacuum, the sample was then transferred, and the same procedure was followed as with the green material.

In order to determine whether the treatment had extracted the carotene quantitatively, the values obtained were checked against those secured by a recently published method (*3*) which consisted mainly of grinding the sample in a mortar with sand and refluxing for one hour with alcohol. The remaining part of the procedure was identical with that outlined above. The results of these comparisons are shown in Table I.

Table I. Carotene Values

(Obtained by refluxing with alcohol for 1 hour or extracting in the blender for 5 minutes)

Sample	Alcohol Extraction	Blender Extraction
	Micrograms per gram	
Fresh green material		
June grass	153.0	158.0
Clover	195.0	202.5
Oats	130.0	130.0
Alfalfa leaves	97.0	105.0
Rye	100.0	102.0
Dock leaves	135.0	130.0
Dandelion leaves	80.0	75.0
Carrot tops	135.0	141.0
Cabbage-weed leaves	131.0	135.0
Plantain leaves	100.0	100.0
Clover leaves	169.0	170.0
Carrot root	103.0	109.0
Silages		
Alfalfa-brome grass	42.5	40.5
	30.3	31.0
Sweet clover	5.5	5.0
	5.3	5.0
Corn	4.5	4.8
	1.0	1.3
Alfalfa	42.3	36.8
	32.5	31.0
	25.0	24.5
Hays		
Alfalfa	11.3	12.0
	5.5	6.0
	7.5	7.8
	24.5	25.0
	7.5	8.0
	13.0	14.5
	1.8	2.1
Soybean	30.0	30.5
	82.0	82.0
	62.5	62.5
	51.0	54.0
	53.0	54.0
Dehydrated alfalfa-leaf meal	61.0	61.0
	64.0	63.0

Discussion

The blender with the foaming mixture extracted the carotene as effectively in 5 minutes as refluxing the sample with alcohol for 1 hour. The extraction method has another advantage in that it is not necessary to grind the silage or green samples in a mortar previous to extraction, and furthermore, in the case of fresh green material enzyme destruction of the carotene is minimized because of the short time required to macerate the sample.

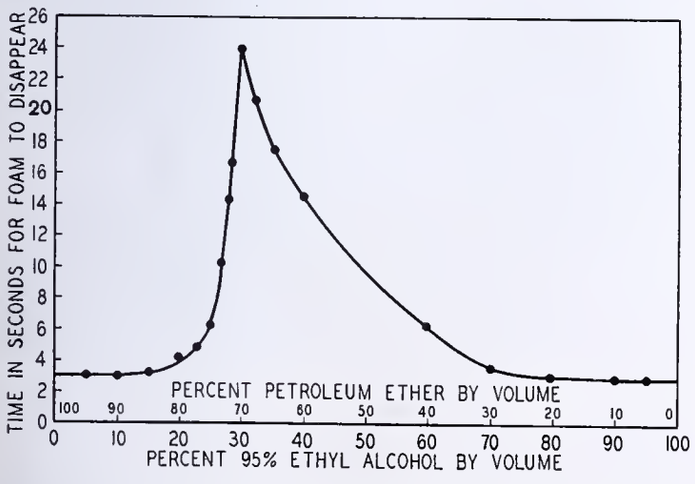

Figure 1. Relationship between Stability of Foam and Concentration of 95 Per Cent Ethyl Alcohol and Petroleum Ether

In making carotene determinations by this method certain precautions are necessary. The concentration of water must be so limited that the two solvents remain miscible with each other. As soon as the addition of water makes them immiscible the foaming tendency disappears and it is often necessary to add more alcohol to the mixture. Although it was not tried, the use of absolute alcohol might obviate to a large extent the addition of more alcohol. The method works especially well where chromatographic separation is to be made on the extract.

The dried samples must be wet with water before extraction where the sample is not finely ground, probably because the mixture is not able to wet entirely the inside of the large stemmy particles. The water apparently wets the particles and is replaced by the solvent during extraction.

Figure 1 shows that there is considerable latitude in the concentrations of alcohol and petroleum ether possible, since a mixture which foams for a greater period than 4 to 4.5 seconds is usable. However, concentrations of petroleum ether greater than 43 per cent by volume were not tested for their ability to extract carotene.

There is some indication that the mixture of the two solvents is a better solvent than alcohol by itself—for instance, only small amounts of lycopene were extracted from tomatoes with alcohol, while the mixture was quite effective.

Experiments with mixtures of other solvents indicated that the foaming tendency was largely limited to these two solvents.

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Rapid Determination of Phosphorus in Ferromolybdenum and in Calcium Molybdate

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TWO methods have been given for the determination of phosphorus in ferromolybdenum (*2*).

In the first method, the solution of the alloy is treated with ammonium or sodium hydroxide, to remove most of the molybdenum. Two separations are usually suggested; if only one separation is used a long process of washing is required. With the bulk of the molybdenum removed, the iron precipitate can be dissolved and the phosphorus precipitated with ammonium molybdate, as in steels. In a second method, the phosphorus is separated as magnesium ammonium phosphate in the presence of citric acid. This method is no more satisfactory for phosphorus than is the separation of magnesium under the same conditions. Precipitation is slow (overnight) and not always complete.

With calcium molybdate, ferric chloride can be added to the solution of molybdate with subsequent separation by ammonium hydroxide, as in the ferromolybdenum method.

Phosphorus in ferromolybdenum and in calcium molybdate is determined (without previously filtering off any compounds) as the yellow phosphomolybdate.

This paper presents a more rapid routine method for the determination of phosphorus in ferromolybdenum and in calcium molybdate.

Experimental

FERROMOLYBDENUM. A 1-gram sample was transferred to a tall form 300-cc. beaker, 10 cc. of (1 + 2) nitric acid were added, and the beaker was immediately covered with a watch glass. After the first rapid reaction, the beaker was placed on a warm plate to complete solution of the alloy, then removed from the plate and hydrofluoric acid was added (10 drops for low and 20 drops for high-silicon alloys). Then 10 cc. of perchloric acid (70 per cent technical) were added, and the beaker was set in a "no-bump beaker" holder on the hot plate. The cover was removed to distill out the nitric and hydrofluoric acids, then replaced. Heating was continued until considerable amounts of molybdenum trioxide separated. Poor results were obtained on those samples which were not fumed well.

The beaker was cooled, and about 25 cc. of water were added. From a graduate, ammonium hydroxide (specific gravity 0.9) was added. A volume of 15 cc. was found satisfactory to dissolve all the trioxide and precipitate ferric hydroxide. Another 25-cc. portion of water was added to cool the mixture. A thermometer was placed in the beaker, and in order to keep the temperature below 40° C. the reaction beaker was placed in a larger beaker containing cold water. Nitric acid (specific gravity 1.42) was added from a buret, and the amount of acid required to dissolve the ferric hydroxide was noted. Measured excess portions of nitric acid (6, 8, 10, and 12 cc.) were added to a series of beakers to determine the optimum volume of nitric acid, 50 cc. of molybdate solution were added at once, and the mixture was stirred several minutes, or put in a shaking device. The cooling bath, which was now warm, was not removed (for hand stirring) because it accelerated separation of the yellow precipitate. The time of settling and the character of the precipitate were noted. The precipitate was filtered and titrated as usual by the alkalimetric method.

Since no standard ferromolybdenum was available, a synthetic mixture of 0.5 gram of standard steel and 1 gram of ammonium molybdate was used to check the composition of the precipitate.

CALCIUM MOLYBDATE. A 1-gram sample was used. The solvent was 2 to 1 hydrochloric acid (*3*) plus one drop of nitric acid. Heat was applied till only a small residue was left. From this point, the same procedure was followed as for ferromolybdenum.

Procedure

FERROMOLYBDENUM. Transfer a 1-gram sample to a tall 300-cc. beaker, add 10 cc. of 1 to 2 concentrated nitric acid, cover with a watch glass, and heat, if necessary, to effect complete solution. Remove from the hot plate, add about 10 drops of hydrofluoric acid (20 drops for high silicas) and 10 cc. of 70 per cent (technical) perchloric acid, place in a no-bump beaker, heat to heavy fumes, and maintain for about 5 minutes.

Cool, add 25 cc. of water, shake, add 25 cc. of ammonium hydroxide (specific gravity 0.9), and stir to dissolve all yellow precipitate. Add 25 cc. more of cold water, place the reaction beaker in a larger beaker of water, add concentrated nitric acid (sp. gr. 1.42) until the ferric hydroxide is dissolved (approximately 20 cc.), and add just 10 cc. excess of acid. While adding the acid, the temperature of the solution should not pass 45° C. At once, add 50 cc. of molybdate solution (*2*), stir or shake, let stand 2 hours, filter, wash, and titrate as usual with standard acid and alkali (0.148 *N*). 1 cc. = 0.02% P on a 1-gram sample.

CALCIUM MOLYBDATE. Transfer a 1-gram sample to a tall 300-cc. beaker, add a mixture of 15 cc. of 2 to 1 hydrochloric acid and 1 drop of nitric acid, and heat to nearly complete solution. Remove from the hot plate, add about 20 drops of hydrofluoric acid and 12 cc. of 70 per cent (technical) perchloric acid, place in a no-bump beaker, and heat to heavy fumes.

Complete as in the second paragraph for ferromolybdenum, noting that ammonium hydroxide yields a white precipitate with calcium molybdate instead of the red iron precipitate with ferromolybdenum.

Discussion

The obvious way to determine phosphorus in ferromolybdenum is to dissolve the sample in nitric acid and precipitate with molybdate. The fact that standard texts do not present this method indicates that it has not been entirely successful.

The problems are: to take care of interfering elements, to prevent the separation of free molybdic acid, and to form the easily filterable "yellow precipitate".

Of the interfering elements, silica and insoluble silicates are taken care of by hydrofluoric acid. Organic matter is oxidized by nitric and perchloric acids. Vanadium, present in only small amounts, is taken care of by the relatively high nitric acid content (*1*). Likewise, the small amounts of tungsten are converted to ammonium tungstate by the excess ammonium hydroxide, and upon acidification neither retard precipitation of phosphorus nor precipitate as tungstic acid in this large volume of solution (150 cc.). Precipitation at 40° C. eliminates interference by arsenic.

To prevent the precipitation of free molybdic acid, note was taken of the technique in the preparation of the "stock molybdate solution" (*3*)—i. e., dissolve molybdic acid in an excess of ammonium hydroxide and water, then acidify in the presence of considerable excess of ammonium ion, and maintain an excess of 10 per cent nitric acid by volume.

Under these conditions of acidity and of concentrations of ammonium nitrate and perchlorate the yellow precipitate which formed was dense and settled within 2 hours. The color was not reddish, as would be the case if vanadium were carried down. Additional precipitate did not form overnight showing that free molybdic acid was not separating.

The above remarks also apply to calcium molybdate, for after solution in hydrochloric acid, addition of hydrofluoric acid, and subsequent fuming with perchloric acid, the material is found in the same state as the ferromolybdenum.

One precaution must be emphasized. After the sample has been fumed with perchloric acid, the beaker is removed

Table I. Determination of Phosphorus in Synthetic Samples

Ferromolybdenum	
0.025, 0.026, 0.028%	(Si 0.64%)
0.026, 0.027, 0.028%	
0.025, 0.027, 0.027%	
0.028, 0.030%[a]	(Si 0.77%)
0.036, 0.038%	(Si 0.90%)
Calcium Molybdate	
0.014, 0.015, 0.017%	

[a] Manufacturer's analysis: P 0.028%, Si 0.74%.

from the hot plate and cooled, and 25 cc. of water are added, followed by 25 cc. of ammonium hydroxide. The mixture must be stirred to dissolve all the molybdic acid. Failure to do so leads to high results. Previously, solid ammonium nitrate had been used (because of its cooling effect) in conjunction with less ammonium hydroxide, but the technique was changed to include 25 cc. of ammonium hydroxide to be certain that no deposits of oxide remained.

The purpose of the "synthetic sample" is to check the yellow precipitate, and to show that the standard sodium hyhroxide–hydrochloric acid solutions should be 0.148 *N* as with the usual precipitations for phosphorus at 40° C. Ordinary yellow precipitate, $(NH_4)_3PO_4.12MoO_3$, requires 23 moles of sodium hydroxide for each mole of phosphorus P; but when precipitation takes place at about 80° C. the yellow precipitate is $(NH_4)_3PO_4.12MoO_3.HNO_3$, which requires 24 moles of sodium hydroxide per mole of phosphorus. Thus, the fact that there exists more than one yellow precipitate requires proof as to whether or not a new yellow precipitate forms under the new conditions of precipitation. The results indicate that the familiar $(NH_4)_3PO_4.12MoO_3$ forms.

Two previous papers (*4*) have discussed the determination of sulfur and copper in ferromolybdenum.

Results

The first synthetic sample was 1 gram of ammonium molybdate plus 0.5 gram of Bureau of Standards No. 82 (0.102% P, 2.78% C, 2.09% Si), planned to represent a high-silicon high-carbon ferromolybdenum. This represented 0.051 per cent phosphorus. The recovery was 0.051 and 0.051 per cent. A second synthetic sample used Bureau of Standards No. 19c (0.049% P, 0.21% C, 0.20% Si). This represented 0.025 per cent phosphorus. The recovery was 0.023 and 0.026 per cent phosphorus.

Results for routine determinations are found in Table I.

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Determination of Zirconium in Steel

A Rapid Colorimetric Method

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THE authors, needing a rapid routine method for the determination of zirconium in steel and finding none that was completely satisfactory, made a search of the general literature on zirconium determinations. After investigation of several methods, the precipitation of zirconium by *p*-dimethylaminoazophenylarsonic acid described by Feigl (*1*), together with the colorimetric measurement of the precipitated reagent as reported by Nazarenko (*2*), was selected as the most satisfactory approach.

This determination can be made without preliminary separation of iron or other elements, although considerable amounts of manganese, silicon, chromium, etc., are present in the steel. This, however, necessitates standardization against similar steel of known zirconium content. The importance of this is shown in Figure 1, which was obtained by plotting milligrams of zirconium against the readings of a Cenco-Sheard-Sanford photelometer on semilog paper.

When no considerable amount of other metals is present, and the solutions to be precipitated contain more than 5 micrograms in 10 ml. or less of 2 *N* hydrochloric acid, line *C* is obtained. The precipitate in this case probably contains the reagent and zirconium in the proportions of 2 to 1, as indicated by $[(CH_3)_2N\langle\bigcirc\rangle N = N\langle\bigcirc\rangle AsO_3]_2Zr$.

When the solutions contain the same amount of zirconium in 50 ml. of 2 *N* hydrochloric acid, line *B* is obtained. In this case, much of the zirconium probably precipitates as $[(CH_3)_2N\langle\bigcirc\rangle N = N\langle\bigcirc\rangle AsO_3]ZrO$. Line *A*, which is used with the proposed method, was obtained by precipitating the zirconium from 50 ml. of solution containing 0.1 gram of steel, which had been analyzed for zirconium by the selenious acid method. If extreme accuracy were desired, a preliminary separation of zirconium by means of a mercury cathode or other suitable method would make it possible to use line *C*, giving about twice as many scale divisions for the same amount of zirconium.

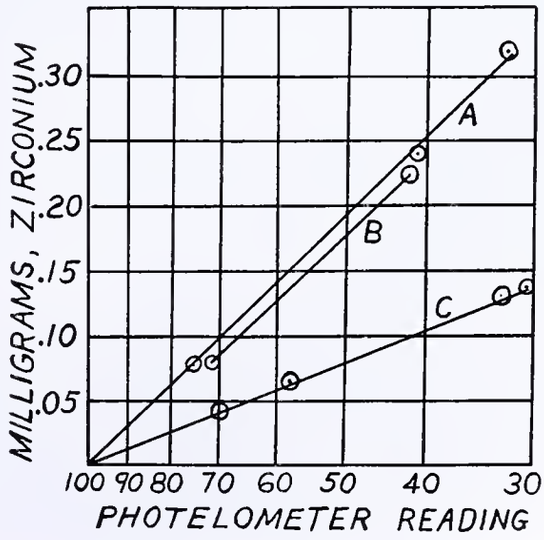

Figure 1

Reagents Required

Hydrochloric acid, 1 part to 1 part of water. Crystalline potassium hydrogen sulfate. Ammonium hydroxide, specific gravity 0.90. Hydrochloric acid wash, 10 ml. of concentrated hydrochloric acid per liter of water. Ammonium hydroxide wash, 333 ml. diluted to 1 liter with water. *p*-Dimethylaminoazophenylarsonic acid, 0.250 gram of dye, 10 ml. of concentrated hydrochloric acid, diluted to 250 ml. with ethyl alcohol.

Method

Dissolve 1 gram of sample in 50 ml. of 1 to 1 hydrochloric acid. When solution is complete, dilute to 75 ml. with water and filter. Save the filtrate. Wash the residue 3 times with hot water, 3 times with hot 1 to 1 hydrochloric acid, and 3 times with hot water. Ignite paper and residue in a platinum crucible, fuse ash with as little potassium hydrogen sulfate as needed, dissolve cooled melt by heating the crucible, and melt in a beaker containing 100 ml. of water and a few drops of sulfuric acid. Now remove the crucible, washing well with hot water, and to the solution of dissolved melt add 2 drops of methyl orange indicator and a slight excess of ammonium hydroxide; boil a minute, filter, and wash the precipitate with cold water. Put paper and precipitate back into the beaker, add 50 ml. of 1 to 1 hydrochloric acid, break paper into pulp, and heat to boiling. Boil a minute and filter into original filtrate saved from first filtration.

Wash well with hot water, transfer to a 500-ml. volumetric flask, cool, and dilute to the mark with water. Mix well and pipet 50 ml. of the combined filtrates into a 250-ml. beaker. Bring the measured 50 ml. of solution to the boiling point, add 15 ml. of dye solution, cover with a watch glass, boil 1 minute, and remove from plate. Let stand at room temperature for at least 30 minutes. Filter through 3 close-texture filter papers, wash excess dye from papers, and precipitate with hydrochloric acid wash solution. After washings are absolutely colorless, set the funnel containing washed paper and precipitate into the neck of a 100-ml. volumetric flask. Now remove dye combined with zirconium by washing with ammonium hydroxide wash, about 3 to 4 washes. Dilute to the mark with water, mix well, and fill absorption cell by filtering through cotton. Photoelectric reading is read in milligrams of zirconium on plotted curve. In this case, weight in grams $\times$ 10 $\times$ 100 = per cent zirconium. The solution of dye which is colorimetered is yellow. A blue filter is used in the photelometer.

In the authors' laboratory this determination is made in less than 2 hours. The results do not deviate by more than 0.005 per cent from those obtained by the selenious acid method.

This method is subject to the same necessity for fusion of the insoluble zirconium with potassium pyrosulfate that is met in all other methods. It is possible, however, to eliminate the usual sodium carbonate fusion when working with steel containing less than 0.05 per cent of phosphorus. The authors have added phosphorus to the original solution of the steel in amounts equivalent to 0.170 per cent without producing any significant change in the results for zirconium. However, with some steels a sodium carbonate fusion of the residue should be made before fusion with potassium pyrosulfate.

Titanium interferes with this method by reacting in the same manner as zirconium, but this can be prevented by adding 3 drops of 3 per cent hydrogen peroxide before the precipitation, if the titanium is not more than 10 times the zirconium content.

The *p*-dimethylaminoazophenylarsonic acid obtained from the Paragon Testing Laboratories, Orange, N. J., has been found very satisfactory.

The authors were handicapped in their work by the fact that there is no Bureau of Standards steel sample of certified zirconium content.

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A Study of the Ferric Thiocyanate Reaction

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THE thiocyanate method for iron is much used. However, it has some disadvantages. Nitrates generally intensify the color, although small amounts may be tolerated with some concentrations of thiocyanate (*14*, *16*). Phosphates change and decrease the color (*4*, *5*) and salts may interfere (*1*, *3*, *5*, *9*, *13*). In view of these facts a study of the optimum conditions for the development of the red ferric thiocyanate color and the effect of salts on the color was undertaken.

It has been assumed by Schlesinger and VanValkenburgh (*12*) that the red substance is the complex ion, $Fe(CNS)_6^{---}$; however, the work of Bray and Hershey (*2*) and Lamb and Jacques (*6*), indicating the existence of $FeOH^{++}$ and $FeCl^{++}$, leaves the authors ready to accept evidence that the complex may contain less than six thiocyanate ions.

Procedure and Reagents

Dilutions of an iron wire solution, oxidized with hydrogen peroxide, were prepared daily and had a pH of 2.5. The reagents were added in the following order: thiocyanate, dilution water, acid, hydrogen peroxide, iron, and water to the mark. Hydrogen peroxide, when used, was added in quantity to make the solution either 0.0032 or 0.0064 molar. Inasmuch as the formula weights of CNS and Fe are nearly the same, the figures as p. p. m. may be read as molar quantities with only slight loss in accuracy.

Interfering Substances

Nitric acid intensifies the color with thiocyanate in the presence of iron. Experiments in the absence of iron showed that solutions 0.1 *N* with nitric acid also developed a color when the thiocyanate concentration exceeded 0.1 *N* and, if the acid were changed to hydrochloric, solutions 1.26 *N* with this acid developed a color when the thiocyanate exceeded 0.05 *N;* when the hydrochloric acid was weaker, 0.1 *N*, the thiocyanate had to be 0.4 *N* to develop color.

In a previous article (*11*) the amounts of hydrogen peroxide necessary to make a colored substance from thiocyanate were given, but no limit for a safe maximum was set. Further work showed that the peroxide could be increased to 0.0064 *M* without significant error, but greater concentrations intensified the yellow color produced.

Thiocyanate–Iron Influence on Color

From the formula $Fe(CNS)_6^{---}$ one would expect to obtain a colored solution when the molar ratio of CNS/Fe equaled or exceeded 6. In order to find out if the amount of thiocyanate necessary to produce a color bore any relation to this ratio, several experiments were performed in which various amounts of thiocyanate were added to iron in 50-ml. Nessler tubes, the acidity being 0.01 *N*. The least amount of

thiocyanate necessary to develop a color was measured. The results are used to make Figure 1. The experiment was repeated in the presence of hydrogen peroxide, but the findings were not significantly different. It appears that when the ferric ion is 0.1 p. p. m. the thiocyanate must be 60 p. p. m. to

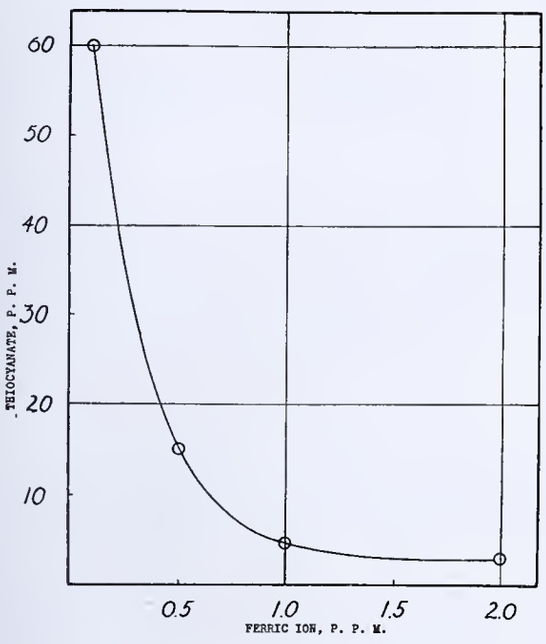

FIGURE 1. AMOUNTS OF THIOCYANATE AND IRON NECESSARY TO SHOW COLOR

show a color; when the iron is 1.0 p. p. m. only 4.5 p. p. m. of thiocyanate are necessary. Apparently, with the higher concentration of iron, only part is active in producing color or else there is a colored substance with less thiocyanate than the postulated $Fe(CNS)_6^{---}$. It is evident that the presence of six ions of thiocyanate to one of iron does not necessarily mean that a colored substance is formed.

Effect of Hydrochloric Acid

In the next series of experiments the effect of varying amounts of hydrochloric acid on the production of the red color was observed. In the first set the iron was 0.5 p. p. m., the thiocyanate 3.0 p. p. m. (ratio 6), and the acid concentration varied from 0.002 to 0.010 *N* in ten tubes; no color was evident. In the second set the iron and thiocyanate were not changed, but the acid concentration was varied from 0.02 to 0.10 *N* in nine tubes; a slight color was present in all experiments, but no difference could be detected between the individual tubes. In the third set the thiocyanate was increased ten times to 30 p. p. m. (ratio 60), and the acid concentration varied from 0.002 to 0.40 *N*. A slight color appeared in all tubes, the strongest being at an acidity of 0.01 *N*. It is evident that most color appears when the acid concentration is 0.005 to 0.010 *N* and that the color decreases when the acid is either less than 0.005 *N* or greater than 0.01 *N*. The data also show that when the CNS/Fe ratio was 6 color first appeared when the hydrochloric acid was 0.02 *N*, while when the CNS/Fe ratio was 60 color first appeared when the hydrochloric acid was 0.002–0.005 and the maximum color appeared at 0.01 *N*. Thus at higher thiocyanate concentrations less acid is required to bring out the color.

It is possible that a change in the amount of iron would cause a change in the acidity requirements. Accordingly the iron was increased to 5.0 p. p. m., and the thiocyanate to 150 p. p. m. (ratio 30). Again most color appeared at an acid concentration of 0.01 *N*, decreasing as the acidity decreased or increased from this figure. The acidity 0.01 *N* thus appears to be the optimum for iron determinations when hydrochloric acid is present. This concentration seems just about enough acid to prevent hydrolysis of the ferric ion; however, the slowness of this reaction, as noted by Lamb and Jacques (*6*), would have the same effect as the presence of more acid. Calculation of the amount of $FeOH^{++}$ existing at a pH of 2, using 3×10^{-3} as the equilibrium constant (*6*, p. 1219), shows that an appreciable amount exists at that pH under equilibrium conditions.

EFFECT OF ACID COUNTERACTED BY THIOCYANATE. The previous results showed that the red color was less intense as the acid concentration was increased beyond 0.01 *N* when the CNS/Fe ratio was 60. To see if this relationship held at higher concentrations of thiocyanate, varying amounts of thiocyanate were added to Nessler tubes containing different amounts of acid at two iron levels and the color intensities were compared. The time from mixing to reading was about 7 minutes. Table I gives the data. It seems that at high thiocyanate concentrations the influence of the acid in decreasing the red color is lessened—for example, changing the acid from 0.01 to 0.60 *N*, when the concentration of thiocyanate was 240 p. p. m. and the CNS/Fe ratio was 2400, resulted in less color in the more acid solution. This held also for a thiocyanate concentration of 1200 p. p. m., CNS/Fe ratio 12,000, but when the concentration of thiocyanate reached 2400 p. p. m. no difference in color was noticed with increased acid. In other words, with a small amount of iron (0.1 p. p. m.), a thiocyanate concentration of 0.040 *N* (2400 p. p. m.) produces the same color whether the acid is 0.01 or 0.60 *N*. When the amount of iron is increased five times to 0.5 p. p. m. it is necessary to increase the thiocyanate concentration to over 11,000 p. p. m., CNS/Fe ratio 22,400, before the effect of the stronger acid is overcome. However, at the two different iron levels at the point where the effect of acid is overcome by thiocyanate, the CNS/Fe ratios are essentially the same. The usual analytical practice is to use a thiocyanate concentration of approximately 0.3 *N* which is, according to this work, high enough to minimize the effect of the acid in lessening color.

TABLE I. EFFECT ON RED COLOR OF VARYING THIOCYANATE CONCENTRATION AT DIFFERENT ACIDITIES

Tube No.	Fe	HCl	CNS	$\frac{Cl}{CNS}$		$\frac{CNS}{Fe}$	Color Intensity
	P. p. m.	*N*	*P. p. m.*	*M*	*P. p. m.*	*P. p. m.*	
1	0.1	0.01	240	2.4	1.5	2,400	<2 but >4
2			1,200	0.48	0.30	12,000	<3 but >5
3			2,400	0.24	0.15	24,000	Same as 6
4	0.1	0.60	240	150	91	2,400	<5 and <1
5			1,200	29	18	12,000	<6 and <2
6			2,400	15	9.1	24,000	Same as 3
7	0.5	0.01	561	1.0	0.65	1,122	<8 but >11
8			1,122	0.52	0.33	2,244	<9 but >12
9			5,610	0.10	0.06	11,220	<10 but >13
10			11,220	0.05	0.03	22,440	Same as 14
11	0.5	0.60	561	62	3.9	1,122	<12 and <7
12			1,122	31	2.0	2,244	<13 and <8
13			5,610	6.2	0.39	11,220	<14 and <9
14			11,220	3.1	0.20	22,440	Same as 10

Considering the Cl/CNS ratio and its effect on the red color, it is found that tube 1, Table I, where the Cl/CNS ratio in p. p. m. was 1.5, had more color than tube 4 where the ratio was 91. Tube 2, with a Cl/CNS ratio of 0.30 had more than tube 5 where the ratio was 18, but in tube 3 with a Cl/CNS ratio of 0.15 the color was identical with that of tube 6 where the Cl/CNS ratio was 9.1 and the amount of acid increased 60 times. It may be concluded that when the thiocyanate concentration is high the chloride ion is less destructive to the red color. Much the same conclusion was reached in the previous paragraph.

Color Increase with Thiocyanate Concentration

The effect of an excess of thiocyanate on the color intensity was next investigated. In the first experiment the solution was 0.01 *N* in hydrochloric acid, the iron was 20 p. p. m., and the CNS/Fe ratio was varied from 1 to 12. The intensity of ratio 6 was taken as standard on the colorimeter. The results are shown in Figure 2. It is evident that color increases linearly as the CNS/Fe ratio increases.

Table II. Effect of Large Excess of Thiocyanate on Red Color

Tube No.	Fe	HCl	CNS	CNS	$\frac{CNS}{Fe}$	Color Intensity
	P. p. m.	*N*	*P. p. m.*	*N*	*P. p. m.*	
1	1.0	0.01	60	0.001	60	
2			120	0.002	120	
3			240	0.004	240	
4			360	0.006	360	Color increases with increase in CNS concentration
5			480	0.008	480	
6			600	0.010	600	
7			1,200	0.020	1,200	
8			1,800	0.030	1,800	
9			2,400	0.040	2,400	
10	0.1	0.01	240	0.004	2,400	
11			360	0.006	3,600	
12			480	0.008	4,800	
13			600	0.010	6,000	
14			720	0.012	7,200	
15			840	0.014	8,400	Color increases with CNS
16			960	0.016	9,600	
17			1,080	0.018	10,800	
18			1,200	0.020	12,000	
19			1,800	0.030	18,000	
20			2,400	0.040	24,000	
21	0.1	0.5	28,880	0.5	288,800	
22			57,760	1.0	577,600	Color increases with CNS
23			86,640	1.5	866,400	
24			115,520	2.0	1,155,200	
25			144,440	2.5	1,444,000	

In the next series of experiments (Table II) the thiocyanate concentrations were increased progressively at different iron and acid concentrations. The color increases with increased thiocyanate concentration in each unit of the series and there seems to be no limit of color intensity that may not come from increasing concentrations of thiocyanate.

Experiments with Acids and Anions

In order to find out if the maximum color effects were due to hydrogen ion and not to the anion, iron salts of several acids were used with thiocyanate in a series of experiments similar to the ones described. The experiment with nitric acid showed that the tubes with acidities ranging from 0.005 to 0.01 *N* had the maximum color. When sulfuric acid or perchloric acid was used, the maximum color developed in the 0.01 *N* acid tube only. Calculations showed that the activity coefficient of the hydrogen ion in these solutions is of the order of 0.9. Apparently the hydrogen ion and not the anion is the effective agent in these low concentration ranges.

To determine the effect of the anion on the color produced, other experiments were made in which equivalent amounts of hydrochloric, nitric, sulfuric, and perchloric acids were used with ferric ion. In all tubes the CNS/Fe ratio was 60 and the iron and acid concentrations were 0.5 p. p. m. and 0.01 *N*, respectively. The solutions were mixed and compared immediately. The depth of color in the chloride, nitrate, and perchlorate tubes varied slightly and in the order $ClO_4^- > NO_3^- > Cl^-$, while the sulfate tube was practically colorless.

Effect of Salts

The initial amounts of reagents necessary to produce a color lasting at least 15 minutes were found to be

$FeCl_3$	0.0000179 *M* (0.1 p. p. m. Fe^{+++})
NaCNS	0.2 *N*
HCl	0.01 *N*
H_2O_2	0.0064 *N* (equivalent to 0.18 ml. of 3 per cent solution in 50 ml.)

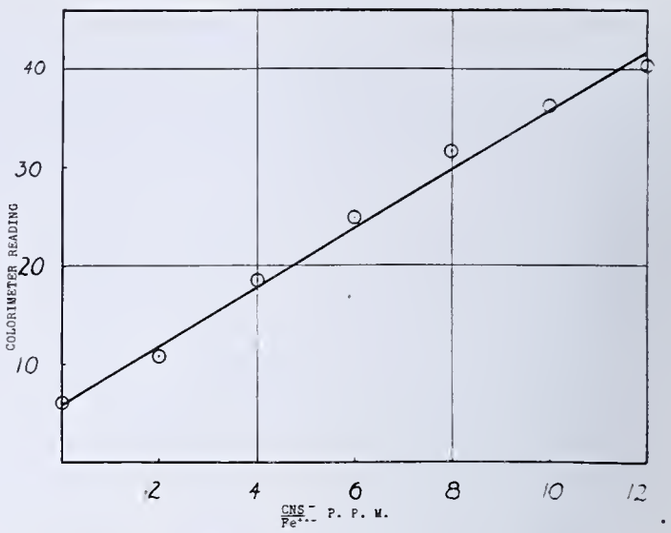

Figure 2. Increase of Color with Increasing Thiocyanate

Acidity 0.01 *N*

Into a series of such solutions were put various salt solutions in the endeavor to find out the least amount of salt necessary to lower the intensity of the color. All solutions were compared with a fresh standard in Nessler tubes. The results are shown in Table III. In the case of all salts studied except the nitrate, the red color was decreased; with the nitrate the color was increased. The amounts of salts necessary to lower color intensity vary widely. Taken in order of decreasing efficiency they are pyrophosphate, monohydrogen phosphate, tertiary phosphate, dihydrogen phosphate, sulfate, chloride, and nitrate. Sodium chloride interfered least with the red ferric thiocyanate color. Table III also shows the salt/Fe and the salt/CNS ratios existing at the point where the salt first causes a lessening in the color intensity. These ratios show that at the point where salt interference began the molar concentration of sodium chloride was 260,000 times that of the iron, while the sodium pyrophosphate was only 0.84 times that of the iron. The values of the salt/iron ratios for the other salts which decreased the red color varied between these two limits.

Table III. Minimum Amounts of Salts Necessary to Reduce Red Ferric Thiocyanate Color Intensity

Salt	Molarity of Salt	$\frac{Salt}{Fe}$ *M/M*	$\frac{Salt}{CNS}$ *M/M*	Color Greater or Less than Standard
$NaNO_3$	0.67	370,000	3.4	+
NaCl	0.46	260,000	2.3	−
Na_2SO_4	0.076	42,500	0.38	−
NaH_2PO_4	0.00065	360	0.0033	−
Na_3PO_4	0.00063	350	0.0032	−
Na_2HPO_4	0.00017	95	0.0009	−
$Na_4P_2O_7$	0.0000015	0.84	0.000008	−

Leeper (*8*) gave values of sodium monohydrogen phosphate and sodium pyrophosphate which interfere in 50 ml. of ferric thiocyanate solution as 100 to 120 mg. and 0.40 mg., respectively, calculated as phosphorus pentoxide. The authors' work shows the values for these same salts as 0.47 and 0.004 mg., respectively. Leeper's solutions were 0.4 *N* with sulfuric acid and 0.25 *N* with ammonium thiocyanate, while the authors' solutions were 0.01 *N* with hydrochloric acid, 0.2 *N* with sodium thiocyanate, and 0.0032 *M* with hydrogen peroxide. This seems to indicate that the increase of acid concentration increases the tolerance of the two phosphate salts mentioned.

Ether Extraction

To see whether the CNS/Fe ratio had any effect on the ether extraction of the red color, 5 ml. of ether were added to 50-ml. Nessler tubes containing varying amounts of thiocyanate and

constant amounts of iron and acid were used. With lesser amounts of thiocyanate, color was observable which would not extract with ether, but as the amounts of thiocyanate increased the colored substance was extracted. The data are shown in Table IV, tubes 1 to 10. Other solutions, tubes 11 to 13, extracted with 7 ml. of ether, showed that color was first extracted at a CNS/Fe ratio of 464. To prove further that ether extracts the colored substance only at higher CNS/Fe ratios, a solution which gave an extractable red color was taken. Part of this solution was diluted to a point where color should not extract and iron and acid were added to match the original concentrations. No color was extractable. The nonextractable color was more brownish than the usual ferric thiocyanate color, but in a Nessler tube, by a human eye comparison, it would be read as iron.

Theory

Water molecules may be present in the ferric thiocyanate complex and may produce hydrogen ions leaving hydroxyl ion in the complex, as follows from the theory of olation (*15*), and also from the work of Bray and Hershey (*2*) and Lamb and Jacques (*6*) which requires that hydroxyl ion change to water below the hydrolysis point.

Table IV. Effect of CNS/Fe Ratio on Ether Extract of Ferric Thiocyanate

(Fe, 1.0 p. p. m.; HCl, 0.01 *N*)

Tube No.	CNS/Fe	Aqueous Layer	Ether Layer
1	300	Colored	No color
2	1,200	Colored	No color
3	1,392	Colored	Pink
4	1,624	Colored	Pink
5	1,856	Colored	?
6	2,088	Colored	Pink
7	2,320	Orange	Pink
8	4,640	Orange	More pink than 7
9	9,280	Orange	More pink than 8
10	92,800	Orange	More pink than 9
11	232	Color	No color
12	325	Color	No color
13	464	Color	Slight color

The production of color by higher thiocyanate ion concentration at lower acidities apparently indicates a contest between the thiocyanate ion and water for a place in the complex. When the thiocyanate ion is high less water would be expected to enter and so less hydrogen ion would be necessary to prevent hydrolysis; consequently a more intense color is seen. This indicates that the pH at which iron precipitates is dependent upon the other ions in solution in addition to hydrogen.

The fact that the most intense color appears at an acid concentration of 0.01 *N* indicates that at lower acid concentrations the hydrogen ion splits off from the water molecules in the ferric-aquo complex. The less intense color at high concentrations of acid is to be expected from the salt effect. Further, the action of acid may show itself in reducing the ionization of thiocyanic acid. Latimer (*7*) gives the value of *K* for thiocyanic acid as 1×10^{-4}. Consequently, the increase of hydrochloric acid should be effective in reducing the thiocyanate ions available for complex formation and thus be part of a mechanism in which increased acidity produces less of the colored substance. However, the less intense color at higher concentrations of hydrochloric acid may be due also to the chloride ion getting a position in the complex in the place of thiocyanate ions; that such action is probable is inferred from the work quoted (*6*), which gives evidence of the existence of $FeCl^{++}$. At higher concentrations of thiocyanate ion the chloride ion would be expected to have greater difficulty in forcing its way into the complex and so it is possible to explain the fact that the lesser amount of colored substance, produced when the chloride-ion concentration is high, is increased when the concentration of thiocyanate ion is greater. The action of salt ions in lessening the color is similar; each forces its way into the complex according to its individual tendency in competition with the thiocyanate ions. The explanation of the descending order of effect on color of the series perchlorate, nitrate, chloride, sulfate, would lie in the fact, as stated by Bray and Hershey (*2*), that chlorates and nitrates do not form complexes with iron while chlorides may form $FeCl^{++}$. The formation of sulfate complexes is commonly assumed. The more iron is used up to form other complexes the less is left for union with thiocyanate.

The increase in color intensity from a given amount of iron, when the thiocyanate concentration is continually increased, indicates that there is no end to the increase in colored substance. The conclusion from this is that the ferric thiocyanate complex is highly dissociated. That the reaction is so sensitive to concentration changes may be due to the high color intensity of the complex. The practical application is to emphasize the need for the same concentration of thiocyanate in both sample and standard.

The failure to extract the brownish-red colored substance by ether at low thiocyanate concentrations points to a complex of lesser thiocyanate composition not soluble in ether. Evidence that there is more than one colored substance present in ferric thiocyanate solutions has been offered recently by Møller (*10*).

Summary

Acidity is a factor in color development of ferric thiocyanate. In general, the most favorable acidity is 0.01 *N*, larger and smaller amounts of acid giving less color.

Increasing the thiocyanate overcomes the effect of higher concentrations of hydrochloric acid to lessen color intensity; also at higher concentrations of thiocyanate less acid is needed to develop the same color intensity.

Increasing the thiocyanate progressively increases the colored substance with no indication of reaching a maximum.

A variation in amount of iron, over a limited range, does not change the amount of acid necessary for maximum color intensity.

The minimum amounts of several anions which reduce the color intensity of ferric thiocyanate, within the limits of the experiments here cited, are in the order pyrophosphate, primary phosphate, tertiary phosphate, secondary phosphate, sulfate, chloride, nitrate.

The extraction of the red color by ether depends upon the CNS/Fe ratio, and a high ratio is necessary for iron extraction.

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From a dissertation submitted by Chester L. French to the graduate school of Massachusetts State College in partial fulfillment of the requirements for the degree of master of science.

Analysis of Petroleum Oil–Soluble Sulfonic Acid Soaps

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The source and types of petroleum sulfonic acid soaps are discussed, and the analytical methods used by the authors for estimating oil-soluble soaps, or so-called "mahogany" soaps, are described.

PETROLEUM sulfonic acids are produced in the course of sulfuric acid treatments of petroleum distillates, particularly in the manufacture of medicinal white mineral oils, by drastically treating suitable raw stocks with fuming sulfuric acid. The ordinary acid treatment which is often employed in refining lubricating oil stocks does not produce sulfonic acids of good quality, since normally the acid is weaker in strength, and only sufficient acid is used to remove the undesirable components of an aromatic and asphaltic nature and perhaps certain of the sulfur, nitrogen, or similar compounds. In view of the fact that the original petroleum distillates consist of a large number of individual compounds of molecular weights varying over a wide range, the resulting sulfo acids will consist of similarly complex mixtures. Some of the acids remain dissolved in the oil layer; these are called "mahogany" acids. The others are found in the acid layer; these are called "green" acids. In actual practice the acids are not recovered as such, but in the form of their sodium soaps.

The oil-soluble sulfonate salts, or soaps, as they are sometimes called, have frequently been referred to as petroleum sludge soaps or sulfonate naphthenates, but it is believed that this has created confusion, and that the term "mahogany soap" or "petroleum oil–soluble sulfonate" is more correct and should be employed. The mahogany sulfonates have been more important commercially than the water-soluble green sulfonates, because they are effective emulsifiers and help to form unusually stable emulsions of oil and water. They permit the manufacture of mineral oil compositions which emulsify readily, even in hard water. Their emulsifying power depends a great deal upon their purity—i. e., freedom from contamination with inorganic salts—and the purer the soaps the better their emulsifying ability. Depending on the application and the specific properties desired, frequently small amounts of other products, such as glycols, fatty acid soaps, resinates, and fatty sulfonates, are used in conjunction with the petroleum sulfonates to enhance their emulsifying power. Generally, however, less than 10 per cent of a good grade of the purified soap is required to emulsify petroleum oil in water without the use of any accessory emulsifier.

The chemistry of the sulfonic acids is discussed in detail by Ellis (*8*) and Dunstan, Nash, Brooks, and Tizard (*7*). The mahogany or oil-soluble sulfonic acids generally consist of the compounds of higher molecular weight, and according to Schestakoff (*11*) they possess the empirical composition $C_nH_{2n}.6SO_4$ and $C_nH_{2n}.9SO_4$.

The process used at the Bayway Refinery of the Standard Oil Company of New Jersey for the manufacture and purification of the sulfonate soaps is briefly as follows:

After separation of the sludge containing the water-soluble green acids, the "acid oil" layer, consisting of the treated oil, is neutralized with alkali, and the resulting oil-soluble sodium mahogany soap is extracted from the oil with aqueous isopropyl alcohol. The crude soap extract solution thus obtained contains, in addition to the complex mixture of sodium mahogany soaps, a certain amount of mineral oil, an excess of alkali, some of the impurities present in the original alkali, and some sodium sulfite and sulfate. The crude soap solution is then further purified by suitable processes whereby most of the salts are precipitated and a refined soap solution is obtained.

Commercially the oil-soluble sulfonates are available in at least three forms: (1) the so-called crude soap, (2) the refined soap in water, and (3) the refined soap in oil. They are usually not available in the pure soap form, since the dry soap without solvent is for most purposes too viscous to handle commercially. The crude soap, which is obtained by evaporating the crude alcoholic soap solution described above is generally a viscous product containing 1 to 25 per cent of water and from 1 to 10 per cent of inorganic salts. The actual oil-soluble soap content of this product is generally around 50 per cent. The remainder is oil, part of which may have been added to make the material more fluid and to aid in stripping off the solvent and water at temperatures (below 150° C.) which will not cause any decomposition.

The refined oil-soluble soap is fairly pure and generally contains only a trace of inorganic salts. A mixture of the refined soap with 20 to 35 per cent of water is possible when heated to 60° to 80° C., and it is this mixture which is marketed as the refined soap in water. The third form, the refined soap in oil, is the most common, and this consists of a blend of approximately equal parts of oil and the refined soap.

Many of the analytical methods published in the literature refer to the water-soluble sulfonic acids or so-called green acids. Thus, Burton and Robertshaw (*6*) refer to water-soluble sulfonic acids in admixture with naphthenic acids. Analytical methods for such splitting agents or mixtures of mahogany and green acids have been published by von Pilat and Sereda (*10*) who refer at length to the work of Schestakoff,

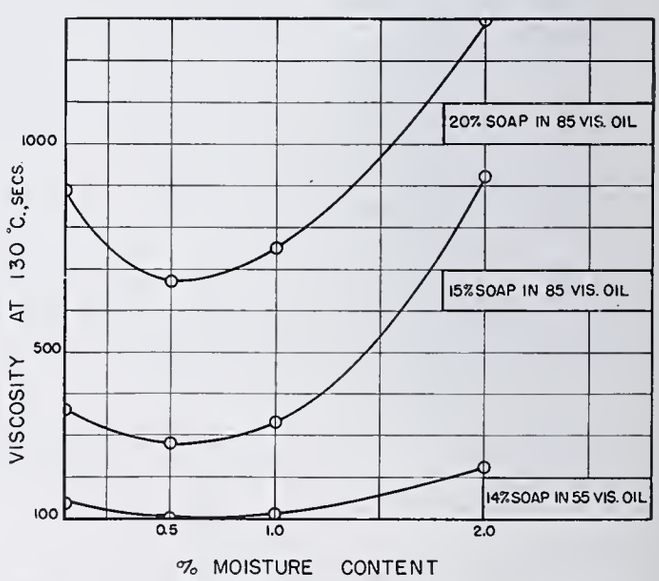

FIGURE 1. VISCOSITY *vs.* MOISTURE CONTENT OF OIL-SOLUBLE SOAP IN OIL

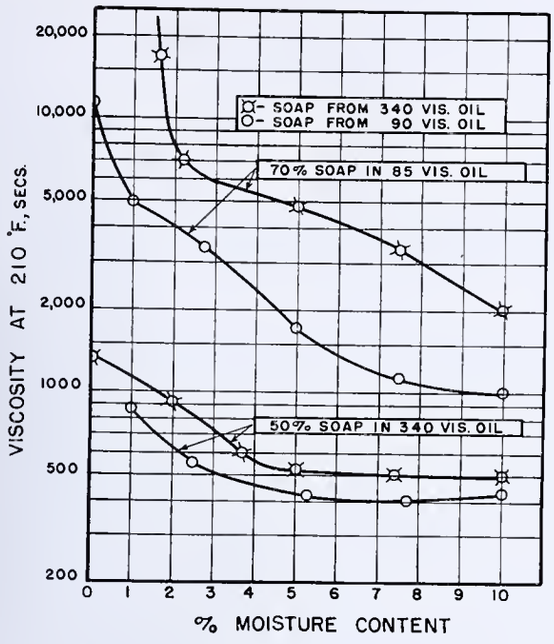

FIGURE 2. VISCOSITY *vs.* MOISTURE CONTENT OF OIL-SOLUBLE SOAP IN OIL

Ilmenjew, and Dobrjanskiij. Such methods are of necessity very complicated, since the separation of both water-soluble and oil-soluble acids, naphthenic acids, and mineral oils is involved. Simpler procedures can be used for the analysis of oil-soluble soaps which are generally free from sulfonated acids of low molecular weight as well as naphthenic acids. The analytical methods described in this paper are limited to the estimation of the soaps of the oil-soluble sulfonic acids.

Methods

Evaluation of the oil-soluble soap is frequently desirable and includes specific gravity, color, viscosity, water content, salt content, oil content, alkalinity, and soap content.

SPECIFIC GRAVITY. The specific gravity is determined most conveniently by warming the sample and mixing it with a mineral oil of 50 to 100 Saybolt seconds at 36.67° C. (100° F.). This lowers the viscosity of the sulfonate, so that the specific gravity may be determined by means of a hydrometer spindle after cooling to room temperature. The gravity of the sample is calculated from the amount of oil added to it and the known specific gravity of this oil used. The specific gravity can also be determined by pouring a heated sample into a standard 100-cc. graduated cylinder, cooling to room temperature, and reading the volume occupied. The weight of the sample is determined by weighing the graduate before and after filling. Either method gives results accurate to 0.5 per cent.

COLOR. The color of crude oil-soluble sulfonate is difficult to determine in the presence of inorganic salts. It is therefore customary to dissolve 20 cc. of the sample in 80 cc. of naphtha, filter through paper, and determine the color of the filtrate with the Tag Robinson or Lovibond colorimeter.

VISCOSITY. The viscosity of oil-soluble sulfonate is of importance as a measure of the pumpability of the material. When it is measured at 99° C. (210° F.) by means of a Saybolt Universal viscometer it is necessary to avoid the evaporation of moisture from the sample, since the viscosity of oil-soluble sulfonate in oil is very sensitive to the moisture content. This is illustrated by the viscosity–moisture content curves of various soap-oil mixtures in Figures 1 and 2. As the soap is hygroscopic, there is also a chance of error where the sample is exposed to humid air at ordinary temperature.

WATER CONTENT. The water content of oil-soluble sulfonate is determined by distilling it with naphtha under a reflux condenser provided with a water trap (*3*). The size of the sample used is adjusted to the capacity of the trap, or for samples of very high water content a trap provided with a stopcock may be used for withdrawal of the water as separated. The latter type of apparatus is valuable where the sample is to be examined for volatile water-soluble materials such as alcohol, amines, or volatile acids. These are readily detected by determining the specific gravity of the water recovered or by titration, etc.

SALT CONTENT. The inorganic salt content is of importance mainly on the crude sulfonate. For an accurate determination, it is necessary to filter out and weigh the salts from the dry naphtha solution of the sample from the moisture determination. Frequently, it is difficult to filter these salts because of their colloidal condition and it is preferable to decant the naphtha solution from the centrifuge and to wash the salts with two portions of petroleum ether, agitating, centrifuging, and decanting the clear supernatant layer at each wash. The salts residue is then washed into a small tared beaker with petroleum ether, evaporated, dried, and weighed.

A more rapid determination of inorganic salts in oil-soluble sulfonate is made by cooling the dry naphtha solution of the sample obtained as a residue from the moisture determination and by centrifuging it in a conical graduated tube. The apparatus and general technique described in A. S. T. M. method D91-35 (*2*) are convenient for this purpose. The reading at the bottom of the tube is converted to per cent by volume based on the weight of the sample used. The practice of reporting inorganic salts (sometimes called "gum") in this manner originates from the fact that crude oil-soluble soap is frequently compounded with petroleum oil to make a fluid product which is later settled to remove the insoluble material and the volume of the sediment represents an actual loss of the product included therein.

OIL CONTENT. The oil content of oil-soluble sulfonate can be approximately determined by dissolving a weighed sample in 50 per cent aqueous isopropyl alcohol and measuring the volume of the supernatant layer of undissolved oil. This is conveniently carried out in a test bottle with a graduated neck and with the aid of a centrifuge to separate the oil layer speedily. The Babcock milk bottle may be used or the A. S. T. M. technique (*4*) is very effective. The rapid and complete separation of the oil is favored by acidifying the alcoholic solvent with sulfuric acid, and isopropyl alcohol has been found to be somewhat more satisfactory than ethyl alcohol because the former is more selective. A 10-cc. sample is used, measured with a standardized pipet which is washed into the bottle with the aqueous alcohol. The tip is broken from the pipet to aid in the filling and subsequent washing. The pipet should then be recalibrated for total content obtainable by washing. For the more viscous soap mixtures the pipet is warmed during use.

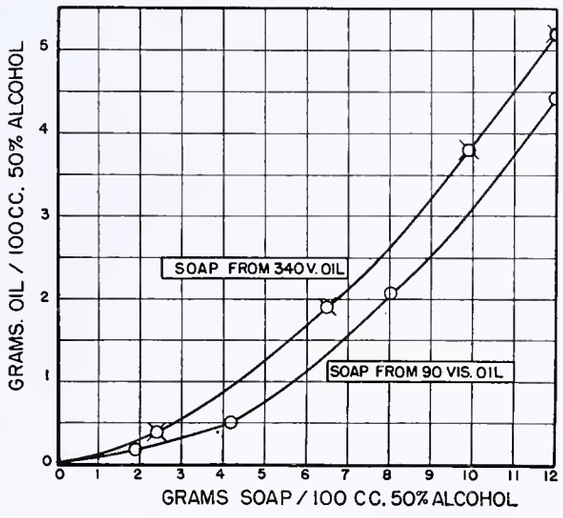

FIGURE 3. SOLUBILITY OF OIL IN SOAP SOLUTIONS IN ISOPROPYL ALCOHOL AT 30° C.

The oil content determination described above is open to the objection that alcohol makes an incomplete separation between the oil-soluble sulfonate and the oil layer. The solubility of the oil in 50 per cent isopropyl alcohol is small but increases rapidly with increasing amounts of oil-soluble sulfonate in the dilute alcohol. This is shown in Figure 3.

The simultaneous direct determination of soap and oil content is accomplished by means of extraction with 50 per cent isopropyl alcohol coupled with counterextraction with petroleum ether. This effectively separates these two ingredients, so that they may be dried and weighed.

Soap Extraction Method. The sample is accurately weighed, taking about 2.0 grams, which are dissolved in 25 cc. of 50 per cent aqueous isopropyl alcohol. The solution is transferred to a 150-cc. stoppered separatory funnel and thoroughly shaken with 25 cc. of petroleum ether, the extraction being carried out at room temperature. The pressure in the funnel is occasionally released by turning the funnel upside down and gently opening the stopcock. (The petroleum ether method for the extraction of unsaponifiable matter in animal and vegetable fats and oils after saponification and solution of the soap in 50 per cent alcohol is official with the American Chemical Society, *1*, American Oil Chemists' Society, *1*, and the Association of Official Agricultural Chemists, *5*.) On standing, the liquid will separate almost immediately into two layers. The lower alcohol layer is drawn into another 150-cc. separatory funnel and extracted again with petroleum ether, while the upper petroleum ether layer is extracted with 50 per cent alcohol, a number of such extractions being carried out until most of the mineral oil is concentrated in the petroleum ether fractions and the soap in the 50 per cent alcohol fractions.

When this analysis is carried out as a routine method, its accuracy is increased by limiting the number of operating steps to the minimum required for consistent results. The following flow diagram gives a satisfactory procedure:

The equipment required is two 150-cc. separatory funnels set in rings one above the other on a single stand. The petroleum ether and 50 per cent isopropyl alcohol are measured out by means of a 25-cc. graduate, and two 150-cc. beakers are used to collect the intermediate washings as well as to hold the final fractions to be evaporated and weighed. The separatory funnels and stoppers are rinsed with 50 per cent alcohol at the close of the test and the washings are added to the alcoholic extract beaker. The portions of alcohol are marked S_1, S_2, etc., and the portions of ether X_1, X_2, etc.

Operation	50% Isopropyl Alcohol	Petroleum Ether
1	Sample + 25 cc. of alcohol = S_1	.....
2	.....	S_1 + 25 cc. of petroleum ether = X_1
3	.....	S_1 + 25 cc. of petroleum ether = X_2
4	X_1 then X_2 + 20 cc. of alcohol = S_2	.....
5	X_1 then X_2 + 20 cc. of alcohol = S_3 S_1, S_2, and S_3 are combined	....
6	.....	S_1, 2, 3 + 20 cc. of petroleum ether = X_3
7	.....	S_1, 2, 3 + 20 cc. of petroleum ether = X_4
8	X_3 then X_4 + 20 cc. of alcohol = S_4	.....
9	X_3 then X_4 + 20 cc. of alcohol = S_5	.....

Oil. Combine X_1, X_2, X_3, X_4, and X_5, remove petroleum ether on steam bath, and dry to constant weight at a temperature not higher than 130° C. Calculate the percentage of mineral oil from the amount of sample taken.

Soap. Combine alcohol solutions S_1, S_2, S_3, S_4, and S_5, and evaporate to dryness to constant weight at a temperature not higher than 130° C. The residue is the sodium sulfonate which can be expressed in percentage from the weight of the sample.

Of the nine operations given above, it is possible to omit Nos. 5 and 9 without appreciably affecting the final results. Any increase in operation beyond the 9 shown has not been found to be justified.

In the case of refined soaps, the oil content plus the soap content determined by this technique can be added to the moisture content to give very close to 100 per cent, as shown in Table I. The salts present in crude sulfonate are included in the weight of the soap extracted according to this method. It is determined as mentioned above and is deducted from the soap found.

A comparison between the analyses of refined oil–soluble soap by the rapid approximate method and the regular analysis is given in Table II. The soap content by the regular method is about 94 per cent of that determined by the rapid approximate method.

Alkalinity. The alkalinity of the oil-soluble sulfonate is conveniently determined by titrating the alcoholic solution from the oil determination test with a standard mineral acid, using phenolphthalein as an indicator.

Table I. Typical Analyses of Refined Oil–Soluble Sulfonates

Sample	A	B	C	D	E	F
Oil	36.1	28.8	27.0	26.8	27.0	27.2
Soap	61.6	68.4	70.4	70.7	70.3	70.7
Water	1.4	2.0	2.3	2.3	2.4	2.4
Accounted for	99.1	99.2	99.7	99.8	99.7	100.3

The material balance of these analyses is considered to be good in view of the fact that the oil has appreciable volatility.

Table II. Analyses of Refined Oil–Soluble Soap in Oil by Rapid Approximate and Regular Methods

Sample	Specific Gravity	Water	Approximate Method Soap	Approximate Method Oil	Regular Method Soap	Regular Method Oil
G	0.925	1.4	36.6	62.0	35.8	62.8
H	0.987	2.0	54.0	44.0	50.6	47.4
I	1.035	2.6	74.4	23.0	68.2	29.2
J	1.040	2.3	74.7	23.0	70.4	27.3

When the above analytical methods are used on purified soap mixtures in water or in oil, the determination of inorganic salts is generally omitted, since the sample is clear of suspended matter. A visual inspection of the naphtha residue from the moisture determination should, however, be noted.

The reproducibility of the above analytical tests on various types of soap products is shown in Table III, which gives results obtained from two independent laboratories using the same methods.

The analysis of crude oil-soluble sulfonate by the extraction procedure is sometimes made difficult by the presence of insoluble impurities. In this case the alcoholic solution should be filtered through paper before proceeding with the analysis.

Special Analytical Methods

The extraction procedure described above gives duplicate results generally agreeing within 0.2 per cent on the total sample. However, soaps of certain types of sulfonic acids are appreciably soluble in petroleum ether. It follows that, while the above extraction procedure will give reproducible results, the petroleum ether solution may still contain sulfonated soaps. It is therefore advisable to carry out an ash determination on the separated mineral oil after its final weighing, also on the recovered soap. According to Hart (*9*) ignition of a pure sodium sulfonate will leave sodium sulfate, and half of the organic combined sulfur trioxide will be volatilized, unless excess alkali in some form is present as an impurity which would retain some of the excess sulfur trioxide. It may be assumed that the ash content of the refined soap should be approximately the same as that of the soap dissolved in the petroleum ether washings. Accordingly, a correction for any soap present in the separated mineral oil may be calculated on the basis of the ash content of the mineral oil. Thus, if the purified soap has an ash content of x per cent and the separated mineral oil y per cent, the amount of soap remaining in the mineral oil will be equivalent to $\frac{100y}{x}$. The weight of soap and oil found in the sample is readily adjusted by this quantity to arrive at a more accurate soap and oil analysis. Table IV gives a typical analysis in which the soap and oil analyses of a sample have been corrected for the ash content of the oil.

In the case of sample A, the correction is so small that it could well be neglected. It occasionally happens, however, that as much as 30 per cent of soap may be left in the separated oil. In such cases the correction is necessary.

The purity of the separated soaps may be checked by carrying out total sulfur and ash determinations. If the soaps are

pure sulfonates, the ash should consist entirely of sodium sulfate the sulfur in the ash being equal to exactly half of the total sulfur.

Total sulfur is determined hy fusing 0.5 gram of the purified soaps with sodium peroxide in a Parr homh. The residue is dissolved in water and any insoluhle metallic oxides resulting from the action of the sodium peroxide on the homh metal are filtered off. The filtrate is made acid with hydrochloric acid, hrought to a hoil, and the sulfate ions are precipitated with harium chloride in the usual way. Blank corrections are made for the sulfur content of the reagents used.

The ash determination is carried out hy weighing accurately 2 grams of the purified soaps into a platinum crucihle and igniting gently until the carbon is hurned off. Ignition is then carried out to redness, to constant weight. A drop of sulfuric acid is added to convert any sodium sulfide produced hy the reduction of some of the sodium sulfate hy the carbon; the excess of sulfuric acid is gently expelled and the residue is finally heated to redness to constant weight, some ammonium carhonate being occasionally added to decompose any sodium hydrogen sulfate which may he present. If no sodium salts, other than sulfonates are present, the weight of the ash after sulfuric acid treatment should show not more than a slight increase (2 to 3 mg.) in weight, due to the decomposition of any sodium sulfide present.

Table III. Analytical Results on Soap Mixtures

(Alkalinity expressed as gas-free Na_2CO_3 per 100-cc. sample)

Laboratory	Specific Gravity	Soap and Salts	Oil	Moisture	Salts	Alkalinity	Color, *R*	Viscosity, 210° F.
					Vol. %			
Crude Soap Analysis								
A	1.033	48.7[a]	42.2[a]	2.1	7.0[a]	0.45	4.25	671
B	1.034	48.3[a]	42.7[a]	2.0	7.0[a]	0.412	4.25	728
Refined Soap in Water[b]								
		Soap		Water				
A	1.056	58.4	8.6	33.0	...	...	..	...
B	1.052	58.6	7.9	33.5	...	...	..	...
Refined Soap in Oil[c]								
A	0.987	52.2	46.4	1.6	...	...	..	620
B	0.983	52.0	46.3	1.7	...	...	26	624

[a] Approximate methods.
[b] Oil present in this sample was that which is unavoidably associated with sulfonate during manufacture.
[c] Color given here is % of sample diluted in white oil which matches 9 Robinson color.

Table IV. Correction of Analysis of a Soap Mixture for Ash Content of Oil Recovered

	Sample A %		Sample B %
Oil content found	3.15		3.66
Ash on above separated oil	0.08		1.50
Ash on pure soap	14.00		14.00
Soap content in oil $= \frac{100 \times 0.08}{14} =$	0.52	$= \frac{100 \times 1.5}{14} =$	10.70
Soap content in mineral oil, calculated on original sample $\frac{3.15 \times 0.52}{100} =$	0.52	$= \frac{3.66 \times 10.7}{100} =$	0.39
Corrected mineral oil content (3.15 − 0.02)	3.13	(3.66 − 0.39)	3.27
Direct soap determination by extraction	46.15		46.04
Corrected soap content = (46.15 + 0.02)	46.17	(46.04 + 0.39)	46.43

The ash content of the pure oil–soluble sulfonate can he used for determining the comhining weight of the sulfonic acids. The figure determined by this method agrees closely with that obtained hy titration of the pure acid. The sulfonic acid can he prepared from sodium sulfonate by treating it with dry hydrochloric acid to precipitate the sodium as sodium chloride in a petroleum ether solution of the purified oil-free soap. The clear naphtha solution is then evaporated on a steam bath, and the residue is weighed, taken up in alcohol, and titrated. On one sample of soap the ash gave a combining weight of 487, as compared with 478 by titration of the pure acid.

The ultimate analysis of oil-soluble sulfonate varies with the viscosity of the oil from which it is made. The base stock from which the oil was obtained also affects the analysis (Table V).

Table V. Analysis of Oil-Soluble Soap

Source	C	H	S	Analysis of Ash	Combining Weight From S	Combining Weight From ash
Coastal 85 viscosity/100° F.	67.02	9.08	6.96	Ca 4.60	460	441
Coastal 340 viscosity/100° F.	65.26	9.09	6.26	Ca 3.82	511	492
Hydrogenated 85 viscosity/100° F.	66.55	8.77	7.76	Na 5.41	413	391

Emulsion Tests

Emulsion tests on oil-soluble sulfonate are used to evaluate it for making emulsible oils, as for metal cutting or textile purposes. The presence of impurities such as dissolved inorganic salts adversely affects the emulsibility of sulfonate soaps.

The emulsibility test consists in determining the border line concentration of sulfonate in a mixture with a standard oil which just gives an oil-in-water emulsion. Lubricating oils vary considerably in aromatic and unsaturate content, so that the amounts of soap to emulsify them vary accordingly. Oils having an appreciable neutralization number generally require more soap to emulsify them than neutral oils. Oil-soluble soaps having a high water content should be dried before testing for emulsibility in order to eliminate the effect of that variable.

White oil of about 90 viscosity at 100° F. Saybolt makes a good standard for testing soap emulsions. The soap is carefully measured and mixed with a known volume of oil, which is increased hy stages until the border of emulsihility is attained. In testing for the emulsion, 45 cc. of distilled water are placed in a 50-cc. stoppered graduate, a 5-cc. portion of the mixture to he tested is added, and the graduate is shaken by hand with 20 vigorous strokes and allowed to stand 5 minutes. This is repeated twice and then the graduate is allowed to stand for 0.5 hour. At the horderline of emulsibility no free oil layer should separate and the volume of the upper layer of emulsion cream should not exceed 5 cc.; otherwise a higher concentration of soap should be tested.

It is customary to set limits of emulsibility to be met—for example, a commercial grade of refined soap should pass the above test at 5 per cent concentration of available soap in the white oil solution. The test can be modified to meet practical considerations by substituting a water of known hardness for the distilled water or a petroleum oil of a standard grade for the white oil. The shaking procedure outlined above was adopted since it aided in canceling out various operating variables. The partial emulsification of the sample when added to the water before the shaking is started and the adhering of this primary emulsion to the walls of the graduate tend to affect the final result unless the outlined procedure is followed.

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Colorimetric Determination of Iron with Kojic Acid

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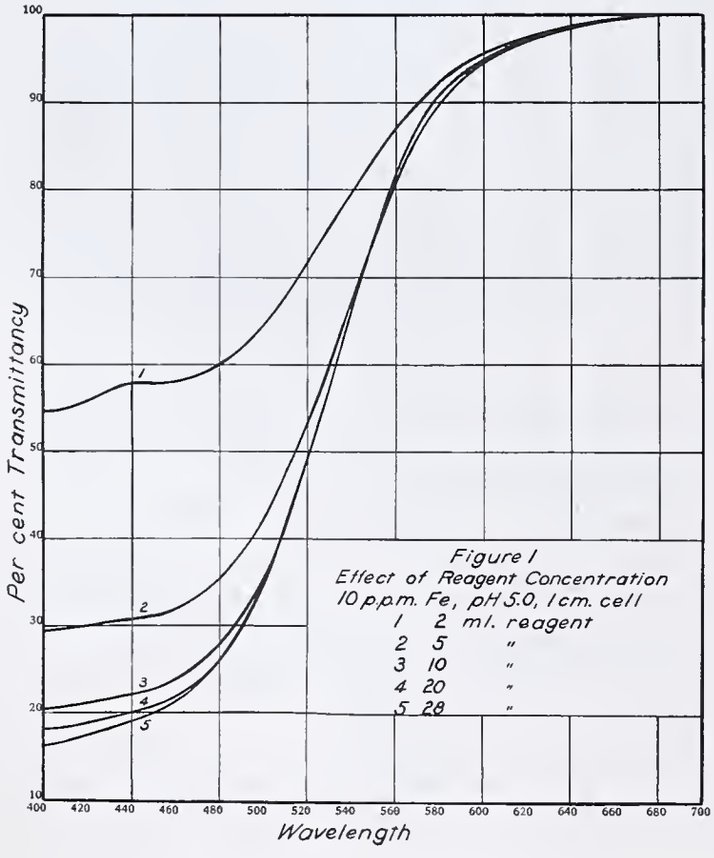
IN A recent paper (*1*) Barham mentioned that Corbellini and Gregorini (*2*) and Tamiya (*6*) had studied the course of certain fermentations producing kojic acid by means of colorimetric determinations of the acid with ferric chloride. It seemed probable, therefore, that this process might be reversed by using the organic compound as a reagent for iron. As tests confirmed this prediction, a spectrophotometric study of the reaction was undertaken in the hope that this new colorimetric method for determining iron would have advantages over existing procedures. Kojic acid (2-hydroxymethyl-5-hydroxy-γ-pyrone) is unique as a reagent for iron, in that no organic compound previously so used is a pyrone derivative.

Apparatus and Solutions

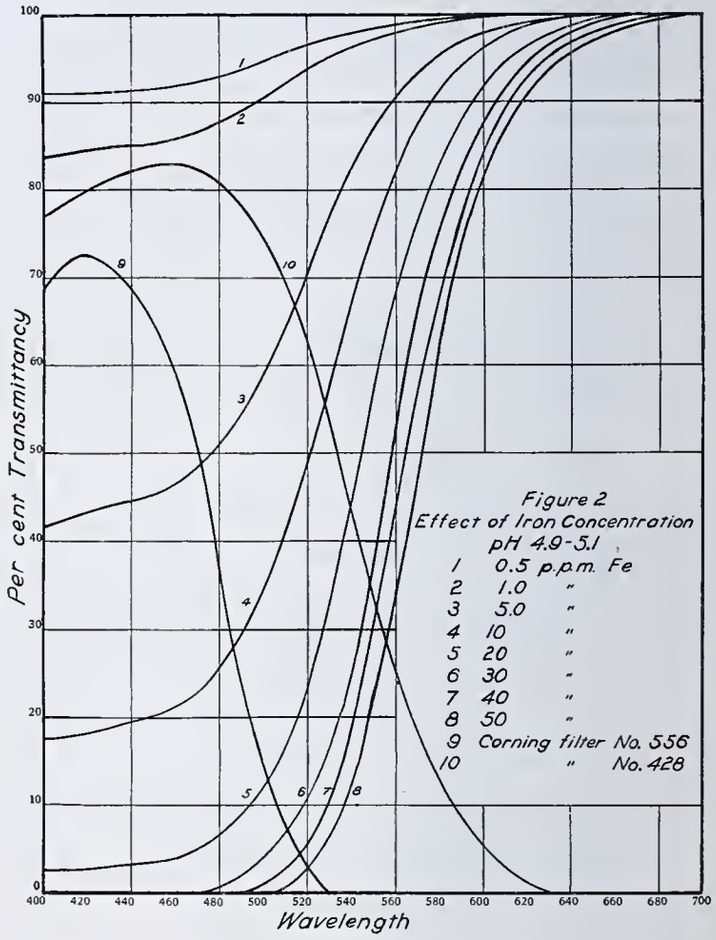
A standard solution containing 0.05 mg. of iron per ml. was prepared by dissolving iron wire of reagent quality in nitric acid and diluting with redistilled water. One milliliter of this solution is equivalent to 1 p. p. m. of iron when diluted to 50 ml.

An aqueous solution containing 0.1 per cent of kojic acid was used as the reagent. More concentrated solutions are susceptible to mold formation on continued standing, although this may be inhibited by using 20 per cent ethanol as solvent or by adding 1 p. p. m. of phenyl mercuric nitrate.

Standard solutions for the study of the effect of diverse ions on the color reaction consisted of alkali metal salts for the anions and of nitrates, chlorides, and sulfates for the cations. These contained 10 mg. of the ion in question per ml. of solution. A solution 1 *M* in ammonium acetate and 0.35 *M* in acetic acid was used for buffering the iron solutions.



In preparing solutions for transmittancy measurements the iron solution was measured and buffered with 10 ml. of the acetate solution. Ten milliliters of the reagent were then added and the solution was diluted to 50 ml. Addition of buffer preceded extraneous ions in determining the extent of interference from these sources. It is recognized that this sequence differs from the situation prevailing in an actual analysis, but it eliminates from the color measurements several undesirable effects, such as precipitation of the iron on addition of basic solutions.



Transmittancy measurements were made in 1.000-cm. cells with a General Electric recording spectrophotometer adjusted for a spectral band width of 10 mμ. A glass electrode assembly (*4*) was used for pH measurements.

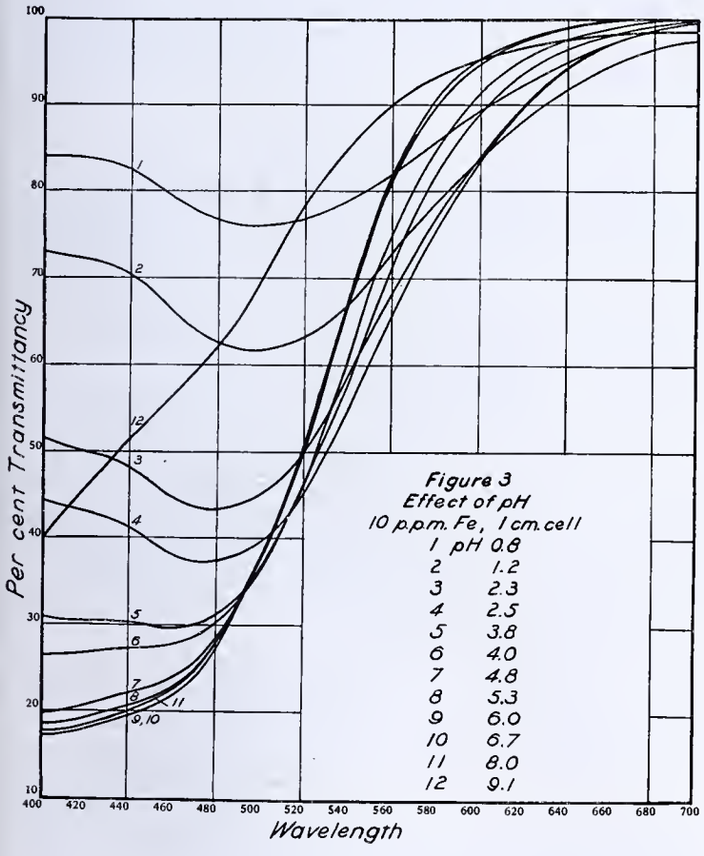
Color Reaction

NATURE OF REACTION. Data obtained according to the method of Vosburgh and Cooper (*7*) indicate that the iron and kojic acid react in a molecular ratio of 1 to 3 at a pH of 5 to 6. It has been shown that the hydroxymethyl group is not concerned in the color reaction, but that the enolic hydroxy is essential (*9*). This suggests formation of a salt with possible implication of the carbonyl group in a five-membered ring. An inner complex salt in accordance with the above ratio would be consistent with the coordination tendencies

of iron, although such nonelectrolytes, as a rule, are insoluble (*3*). Other colored constituents may be present, especially in solutions of lower pH.

Effect of Reagent Concentration. With kojic acid, as with ferron, the intensity of the color developed with iron depends upon the concentration of the color-forming reagent. This effect, shown in Figure 1, necessitates a relatively careful measurement of the quantity of reagent. Ten milliliters of 0.1 per cent solution are sufficient to produce a deep color with 10 to 20 p. p. m. of iron in 50 ml. and little is gained by using excessive amounts.

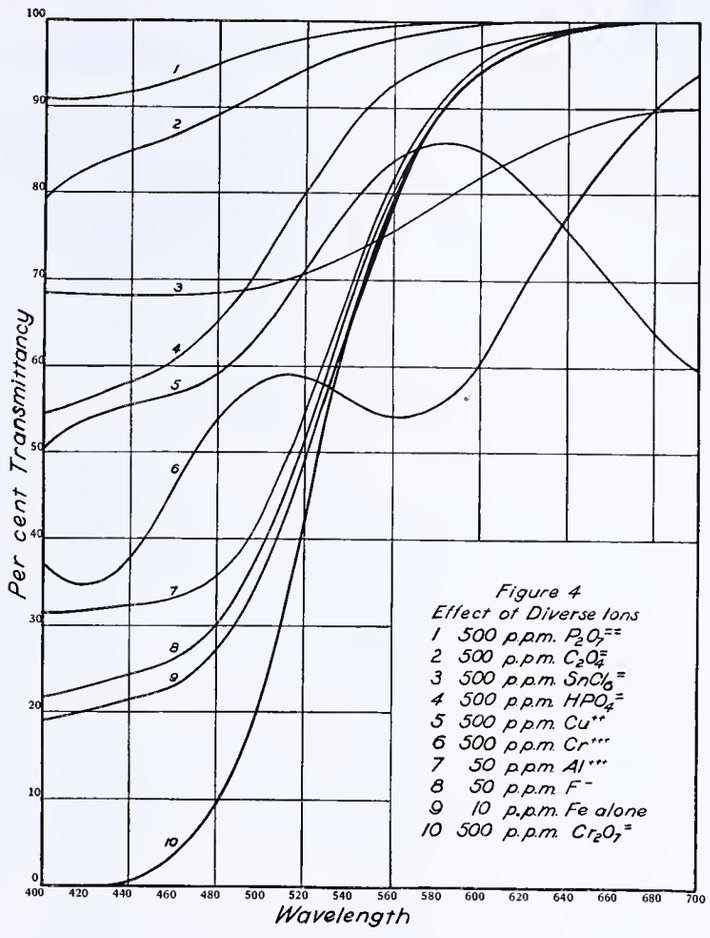
Effect of Iron Concentration. Transmittancy curves for solutions containing from 0.5 to 50 p. p. m. of iron and 10 ml. of 0.5 per cent reagent in 50 ml. of solution are shown in Figure 2. A linear relationship between iron concentration and log transmittancy prevails, in accordance with Beer's law, for concentrations up to about 40 p. p. m. A permanent calibration curve may thus be established by determining the



transmittancy for a given iron concentration and drawing a straight line through the determined point to 100 per cent at zero concentration, on semilogarithmic paper. Conformity to Beer's law should not be a critical consideration in evaluating a method, but it is an advantage in fulfilling the requirements for use of a variable-depth comparator of the Duboscq type. This law is not valid for solutions containing equal amounts of iron but different quantities of reagent.

The concentration range over which the method is applicable depends upon the cell thickness and the means of comparison. One can distinguish 0.05 p. p. m. of iron from a blank in 30-cm. Nessler tubes. Comparison of amounts greater than 5 p. p. m. is difficult. Photoelectric instruments can detect 0.1 p. p. m. in a 1-cm. cell and the upper limit is about 20 p. p. m. The sensitivity is improved by acetone, but the effect is much less than in the thiocyanate method (*8*).

The change of transmittancy with concentration is more pronounced than that of the trichromatic or monochromatic



values, as is usually the case in such systems. The red and green values are practically equal.

Effect of Acid Concentration. As acid concentration exerts considerable influence on the intensity and hue, it should be controlled within rather narrow limits. Strong acids or bases destroy the color, the change in basic solution being irreversible. Figure 3 represents solutions containing 10 p. p. m. of iron and 10 ml. of reagent in 50 ml. of solution at pH values from 1 to 9. The orange hue at pH 5 was stable for more than 5 weeks. A low pH is desirable in order to avoid precipitation of iron, although the tendency to fade increases with the acid concentration.

It is desirable to maintain the pH between 5.5 and 7 during the color measurements, since this range is best for achieving optimum color stability, small variation of transmittancy with pH change, and high absorption in the blue region. Ammonia may be added for adjustment of pH only after development of the color. Otherwise, precipitation of some iron is likely to occur. Ammonium acetate is a convenient buffering agent.

Effect of Diverse Ions (Figure 4). An outstanding disadvantage of colorimetric methods for ferric iron is the fact that certain ions react with the iron to form stable complexes which decrease the ferric-ion concentration. This is encountered especially with phosphates, fluoride, and several organic ions. Other types of interference include formation of colorless complexes by the reagent with various metals, precipitation of insoluble products, presence of colored constituents, and development of a color by the reagent with some ion other than iron. Reduction of part of the iron by certain ions may lead to low results for total iron, although this effect has no relation to the color reaction itself. No reagent free from all these kinds of interference has been reported for ferric iron. Ordinarily, the treatment of the sample is designed to circumvent interference by other substances as

far as possible, but it is desirable to have a reagent which reacts specifically and quantitatively with the desired constituent.

In this study the effect of 500 p. p. m. of the ion in question was determined and, if interference was observed, smaller quantities were used until the change in transmittancy at 440 mμ was reduced to about 5 per cent. The extent of interference can thus be determined accurately in terms of iron concentration. An error of 2 per cent is not prohibitive for colorimetric methods.

Table I. Effect of Diverse Ions

Ion	Added as	Permissible Concentration *P. p. m.*
Al^{+++}	$Al(NO_3)_2$	3
AsO_4^{---}	H_3AsO_4	30
AsO_3^{---}	Na_3AsO_3	170
Be^{++}	$Be(NO_3)_2$	250
Bi^{+++}	$Bi(NO_3)_3$	25
$C_3H_5O_2^{--}$	$(CH_3CHOHCOO)_2Ca$	130
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	50
$C_7H_5O_3^{-}$	$C_6H_4OHCOONa$	500
Co^{++}	$Co(NO_3)_2$	500
Cr^{+++}	$Cr_2(SO_4)_3$	10
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	5
Cu^{++}	$Cu(NO_3)_2$	19
F^{-}	NaF	15
Hg^{+}	$HgNO_3$	100
MoO_4^{--}	$(NH_4)_2MoO_4$	25
Ni^{++}	$Ni(NO_3)_2$	330
Pb^{++}	$Pb(C_2H_3O_2)_2$	250
PO_4^{---}	$(NH_4)_2HPO_4$	20
SiO_3^{--}	Na_2SiO_3	100
$SnCl_4^{--}$	H_2SnCl_4	10
$SnCl_6^{--}$	H_2SnCl_6	5
Th^{++++}	$Th(NO_3)_4$	25
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	200
VO_3^{-}	KVO_3	5
WO_4^{--}	Na_2WO_4	30
Zn^{++}	$Zn(NO_3)_2$	140
ZrO^{++}	$ZrO(NO_3)_2$	5

No effect on the color was observed in solutions containing 500 p. p. m. of each of the following ions: acetate, silver, barium, borate, benzoate, bromide, calcium, cadmium, chloride, chlorate, perchlorate, carbonate, formate, mercuric, potassium, lithium, magnesium, manganese, sodium, ammonium, nitrate, thiocyanate, sulfate, and strontium. Table I shows the maximum permissible quantities of various ions not already mentioned which may be present without causing an error greater than 2 per cent.

Reducing ions, such as cyanide, iodide, nitrite, and sulfite, did not interfere if added to the iron solution after addition of the acetate buffering solution. If the ion was added before buffering the solution at pH 5, however, low results were obtained. Such ions would be oxidized in the normal treatment of the sample and thus present no difficulty.

Low results are caused by certain ions, including phosphates, fluoride, and a few organic acids. Citrate, oxalate, and pyrophosphate must be entirely absent.

The extent of fluoride influence may be applied for determining this element, although the sensitivity is probably too low for the method to be practical in visual work. The relation between fluoride concentration and transmittancy of a solution containing 1 p. p. m. of iron and kojic acid is close to linear up to 100 p. p. m. of fluoride. A change in transmittancy of only 1 per cent corresponds to 6 p. p. m. of fluoride, however, which would necessitate very accurate measurement of the color.

Aluminum, zinc, and a few other metals form colorless complexes with the reagent, thus lowering its effective concentration and leading to low results. This source of interference cannot be eliminated satisfactorily merely by adding more reagent, since the color reaction is not stoichiometric.

All highly colored compounds should be absent unless their absorption bands can be eliminated by proper setting of the monochromator.

Recommended Procedure

Procure a representative portion of the material to be analyzed and subject it to the necessary preparative treatment.

Weigh or measure by volume a quantity of sample containing 1 mg. of iron or less.

Treatment of Sample. Ores may be dissolved in hot hydrochloric acid. Chlorostannous acid should not be added to hasten dissolution unless the solution is to be diluted to 1 liter or more. Organic matter combined with the iron necessitates the use of ashing procedures.

After dissolution of the sample, oxidize the iron by boiling with a little concentrated nitric acid. Other oxidants, such as persulfate or hydrogen peroxide, may be used.

Dilute the oxidized solution with iron-free water and add 1 gram of ammonium acetate. Filter if the solution is not clear. Transfer the solution to a 100-ml. volumetric flask, add 10 ml. of 0.1 per cent kojic acid solution, dilute to the mark, and mix well.

Measurement of Desired Constituent. The color, which is developed immediately, may be measured by the usual methods. Standards for visual comparison, prepared with the same acid concentration as the sample, may be kept for a week. If a filter photometer is used, the curves of Figure 1 indicate that the filter should be a blue or blue-green, such as Corning glasses Nos. 533, 554, and 429.

Summary

A spectrophotometric study of the reaction between iron and kojic acid indicates that this compound is a suitable reagent for the colorimetric determination of ferric iron. Measurements were made on low-iron waters and on ores containing as high as 50 per cent of iron. Values for the ores agreed within a few tenths of a per cent with the titrimetric values and required somewhat less time.

Although this method is not ideal, it is free of some of the interferences of other methods, and the sensitivity is adequate, especially with blue filters in a photometer. The color is relatively stable and conforms to Beer's law over a wide range. The chief limitation of the method is the necessity of controlling the acidity. A variation between pH 5.5 and 7 is permissible, and this range yields an orange hue suitable for visual comparisons. These limits are much wider than those for reagents such as ferron (*5*).

Maximum sensitivity is obtained if the iron concentration is between 1 and 20 p. p. m. for a cell thickness of 1 cm. The color is somewhat less intense than that formed with thiocyanate or *o*-phenanthroline, thus permitting determinations on samples higher in iron than these other methods can accommodate without dilution. The method is not applicable to samples containing aluminum, citrate, oxalate, or pyrophosphate.

Acknowledgment

H. N. Barham of Kansas State College very kindly furnished the kojic acid.

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Abstracted from a thesis presented by M. L. Moss to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of master of science, June, 1940.

The Chemistry of Menhaden Oil

Component Fatty Acids

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OILS of domestic origin are becoming more important by reason of the increasing inability to import foreign oils. Marine oils in particular are available in large quantities and should be given serious consideration as replacements for foreign materials. Because much is still unknown of the chemistry of marine oils, the present investigation has been undertaken. Menhaden oil was chosen for the start of this research, since it is the most abundant marine oil produced on the Atlantic coast.

Table I records some of the published results.

Twitchell (*8*) in 1917 investigated the composition of menhaden oil by the lowering of the melting point of the mixtures of fatty acids which he isolated. Saturated and unsaturated acids were studied separately, the latter after hydrogenation. His results revealed no unsaturated acids with fewer than 18 carbon atoms. Brown and Beal (*2*) fractionated the methyl esters, finding acids of 14, 16, 18, 20, and 22 carbon atoms. Armstrong and Allan (*1*) reported, in addition to those previously found, small amounts of saturated acids with 20 and 22 carbon atoms. Richardson, Knuth and Milligan (*5*) have published ester distillation data and more recently Stingley (*7*) has reported the component fatty acids of menhaden oil (Table I).

TABLE I. COMPONENT FATTY ACIDS OF MENHADEN OIL

Carbon Atoms	Twitchell (*8*)	Armstrong and Allan (*1*)	Stingley (*7*)	This Research
	%	%	%	%
		Saturated Acids		
14	9.2	5.9	7.0	8.3
16	22.7	16.3	16.0	14.9
18	1.8	0.6	1.0	4.7
20	..	0.6	..	..
22	..	0.8	..	..
		Unsaturated Acids		
12	..	..	..	Trace
14	..	..	Trace	5.8
16	..	15.5	17.0	23.4
18	24.9	29.6	27.0	31.1
20	22.2	19.0	20.0	8.4
22	20.2	11.7	12.0	3.4

Commercial menhaden oil from the Atlantic coast was examined by the ester fractionation procedure. Among the saturated acids (Table I) were found myristic, 8.3 per cent; palmitic, 14.9 per cent; and stearic, 4.7 per cent. Myristic and palmitic acids were isolated from the corresponding esters. Attempts to isolate an acid of higher molecular weight than palmitic have ended with mixtures. Fraction 6 yielded a mixture with a melting point of 57.5–58.5° C. and a neutral equivalent of 264.8 which lies between that calculated for palmitic and stearic acids. The neutral equivalent and the freezing point of 55.8° C., according to the data of Shriner, Fulton, and Burks (*6*), indicate a mixture of 0.85 mole of palmitic acid and 0.15 mole of stearic acid. This mixture has a neutral equivalent calculated to be 260.4.

The fractional distillation of the methyl esters of the unsaturated acids and the saponification equivalents of the fractions were used to calculate the composition. As a check upon this method, the acid from fraction 4 was hydrogenated catalytically to palmitic acid.

Analytical Methods

The methyl esters were fractionated through an electrically heated column packed with small helices. On top of the column was fitted a head designed for total reflux with partial take-off.

Saponification equivalents were determined by the method of Chargoff (*3*). The Hanus method was used for the determination of iodine numbers. Neutral equivalents were determined in warm alcohol by direct titration with alkali to a phenolphthalein end point.

Experimental Procedure

Six hundred grams of commercial oil produced by centrifugation were saponified with alcoholic potassium hydroxide and the unsaponifiable matter was removed with ether. The residual soaps were acidified. The fatty acids were taken up in ether, washed with distilled water, and dried with anhydrous sodium sulfate, and the ether was removed. The recovered acids weighed 552 grams, a yield of 92 per cent of the original oil. Double precipitation of the lead soaps in alcohol and liberation by the usual methods yielded 27.9 per cent saturated and 72.1 per cent unsaturated acids.

The methyl esters of the saturated acids were prepared by dissolving in dry methanol and saturating with dry hydrogen chloride gas. After standing at room temperature for 12 hours they were treated in the usual manner. The methyl esters so obtained had a saponification equivalent of 267.4 and an iodine number of 4.0. The esters were fractionated and the data are tabulated in Table II.

Fraction 1 was saponified with alcoholic potassium hydroxide and acidified and the precipitate was crystallized from 95 per cent alcohol. The observed melting point was 53° C., while Francis and Piper (*4*) reported 54.4° C. for synthetic myristic acid. From fraction 3 an acid was separated and crystallized from acetone. The observed melting point was 63° C.; reported for palmitic acid, 62.9° C. (*4*).

The acid isolated from fraction 6 was crystallized from acetone and recrystallized from methanol and had a melting point of 57.5–58.5° C. The observed neutral equivalent was 264.8; that calculated for palmitic acid is 256.3 and for stearic acid is 284.3. The freezing point of this mixture as calculated from cooling curves was found to be 55.8° C. Shriner, Fulton, and Burks (*6*) have studied the cooling curves of many mixtures of palmitic and stearic acids and of the two mixtures reported freezing at 55.8° C.; that consisting of 0.85 mole of palmitic acid and 0.15 mole of stearic acid has a calculated neutral equivalent of 260.5 which comes closer to the observed.

TABLE II. FRACTIONATION OF METHYL ESTERS OF SATURATED FATTY ACIDS

Fraction	Weight	Boiling Range (5 Mm.)	Saponification Equivalent
	Grams	° *C.*	
1	11.1	147–154	244.1
2	9.9	154–160	254.5
3	7.3	160–165	258.2
4	18.7	165–169	279.2
5	12.5	161–163[a]	277.1
6	4.4	170–178	284.9
Residue[b]	5.4	...	...

[a] Difficulty was experienced in maintaining pressure at 5 mm. A new pump and other improvements helped in later experiments.
[b] Dark brown in color.

The methyl esters of the unsaturated acids prepared by the methanolic hydrochloric acid method had a saponification equivalent of 293.7 and an iodine number of 187.4. The data for the fractions obtained from the electrically heated packed column are summarized in Table III.

TABLE III. FRACTIONATION OF METHYL ESTERS OF UNSATURATED FATTY ACIDS

Fraction	Weight	Boiling Range (5 Mm.)	Saponification Equivalent	Iodine No.
	Grams	° *C.*		
1	2.2	120–123	248.5	24.8
2	1.8	123–135	260.0	83.6
3	3.6	135–140	254.7	103.7
4	10.7	140–147	272.0	123.1
5	3.2	147–155	280.0	125.4
6	6.9	155–160	291.0	127.3
7	6.6	160–165	292.0	153.1
8	7.2	165–170	298.0	166.5
9	4.5	170–175	308.0	183.2
10	6.2	175–180	310.0	212.2
11	3.7	180–185	325.0	226.2
12	5.5	185–190	311.5	208.1
13	4.4	190–195	352.0	232.7
Residue[a]	9.3	.....	...	...

[a] Dark brown in color.

To confirm the composition as calculated from the saponification equivalents, the saponification product from fraction 4 was acidified, extracted with ether, and dried, and the ether was removed. Hydrogenation in acetic acid with palladium–barium sulfate catalyst produced an acid with a melting point of 61° C.; that recorded for palmitic acid is 62.9° C. (*4*). The neutral equivalent found was 258.3, while that calculated for palmitic acid is 256.3.

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Reducing Power of Starches and Dextrins

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A rapid ferricyanide method for determining the reducing power of starches and dextrins is presented in which the reduced iron is measured directly by a ceric sulfate titration. For starches hydrolyzed by hot or cold acid or oxidized by alkaline hypochlorite, and for raw starches and dextrins, the reducing power values by this method parallel those determined by the longer procedure of Richardson, Higginbotham, and Farrow. Many other modified starches such as the "chlorinated" and "thin-boiling" types have been measured.

THE copper number has been used extensively for reporting the reducing power of cellulosic materials and recently by Richardson, Higginbotham, and Farrow (*7*) for starch. This paper describes a much simpler method for determining the reducing power or copper number of starches and dextrins, which shortens the time required to less than 25 minutes.

While measuring the reducing power of a series of starches by the Gore and Steele (*2*) modification of the Hagedorn and Jensen (*3*) method, the authors noticed that the apparent maltose equivalent of the more soluble products when converted to milligrams of copper gave values equal to the copper numbers determined by the Richardson, Higginbotham, and Farrow method. For raw starch and very slightly solubilized starch, high values were obtained by the Gore and Steele method. These high values were attributed to the visible entrapment in the starch of iodine which was very difficultly released for measurement in the thiosulfate titration.

In the determination of the maltose equivalent of starch-maltose mixtures Martin and Newton (*6*) avoided the difficulty due to iodine entrapment by using Hassid's (*4*) ceric sulfate titration. It seemed probable that the same ceric sulfate titration could be used to advantage here to measure the reducing power, not of a starch-maltose mixture, but of starch or dextrin alone. This proved to be true. Consideration of the above statements reveals that any reducing value obtained for maltose in the presence of dextrins and modified starches is only an apparent maltose value.

TABLE I. REDUCING POWER OF STARCHES AND DEXTRINS

Sample	R_{Cu}, Mg./Gram
Pearl starch (control)	6.8–7.9
Other commercial cornstarches	7.7, 9.4, 10.1, 11.2, 11.6
Waxy maize starch	9.0
Thin-boiling starch, 40-fluidity	6.5
Thin-boiling starch, 90-fluidity	27.2
Chlorinated starch, 2.5% chlorine	14.9
Chlorinated starch, 5% chlorine	32.9
Electrolytically oxidized starch	6.8, 3.0, 6.3, 4.5, 7.5
Alkali dextrin, A	10.5
Alkali dextrin, B	33.0
Alkali dextrin, C	71.0
Acid dextrin, A	12.0
Acid dextrin, B	25.5
Acid dextrin, C	39.0
Gore (*1*) starch, 5-hour conversion	19.6
Gore (*1*) starch, 42-hour conversion	61.6
Gore (*1*) starch, 96-hour conversion	120.0
Maltose	1900
Glucose	2800

The potentiometric measurement used by Martin and Newton was eliminated because the color change of the solution from green to yellow just before the large voltage change was a satisfactory indication of the end point. However, starch products with very little reducing power produced only a slight green color and made accurate determination of the end point difficult. The addition of a measured amount of glucose solution to each starch sample obviated this difficulty. A correction for the reducing power of this added glucose was made by a blank determination.

When, in the hydrolysis of starch by acid, the R_{Cu} values (milligrams of copper per gram of starch) were plotted against the time, there resulted a straight line for R_{Cu} values up to

1000. This is shown in Figure 1 where the simplified R_{Cu} determination is compared with the longer method of Richardson, Higginbotham, and Farrow. It is evident that the two methods give the same results in the starch and dextrin range but not in the range of the sugars. Figure 2 presents the change of rotatory power, of alkali number (*8*), and of iodine titration (*5*) as the same sample of starch was hydrolyzed. The alkali number became constant before the starch was one third converted to glucose.

This shorter method for copper numbers uses iron salts instead of copper salts, and the results, therefore, might be reported as R_{Fe} units. It seems better not to introduce another such unit, but to report the results as R_{Cu} units to allow comparisons with copper numbers. When the maltose, glucose, or iron equivalent is desired the following conversion factors are used:

$$\text{Per cent maltose equivalent} = \frac{R_{Cu} \times 100}{1900}$$

$$\text{Per cent glucose equivalent} = \frac{R_{Cu} \times 100}{2800}$$

$$R_{Fe} \text{ (mg. of Fe per gram of starch)} = R_{Cu} \times \frac{55.84}{63.57}$$

The utility of the method in the relative characterization of starches and dextrins is shown by Table I. The reducing power increases with increasing conversion of starch products except for the electrolytically oxidized starches, which, because of the method of oxidation and washing, have values equal to or lower than those of raw starches. This simplified determination of reducing power has been used to follow the acid and alkaline conversion of starch—e. g., on the "thin-boiling" starches, on the Gore starches (*1*), and on the dextrins formed by acid or alkaline catalyst (Table I).

Reagents

Alkaline Ferricyanide: 32.9 grams of potassium ferricyanide and 50 grams of anhydrous sodium carbonate dissolved in water and diluted to 1 liter. The solution is approximately 0.1 *N* with respect to potassium ferricyanide.

Glucose Solution: a 0.2 per cent glucose solution containing a small amount of phenol.

Sulfuric Acid Solution (5 *N*): 139 ml. of concentrated sulfuric acid made up to 1 liter of solution.

Ceric Sulfate, 0.05 *N*: 26.5 grams of reagent ceric sulfate (G. Frederick Smith Chemical Co.) added to about 900 ml. of a solution containing 100 ml. of concentrated sulfuric acid. The mixture is digested until the solid has dissolved, cooled, and made up to 1 liter. The ceric sulfate solution is standardized against a standard ferrous ammonium sulfate solution potentiometrically or in the presence of *o*-phenanthroline indicator.

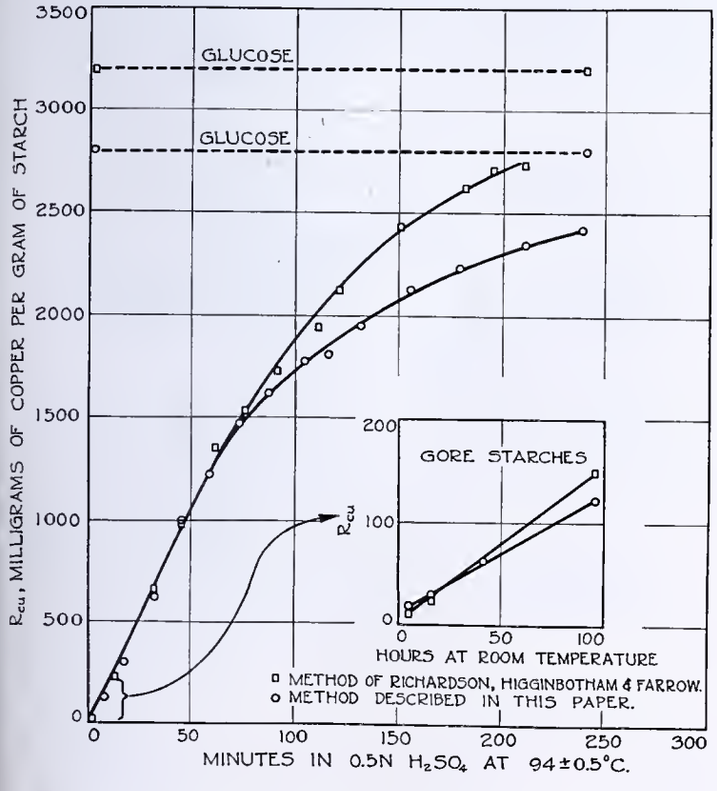

Figure 1. Comparative Determinations of Reducing Power during Hydrolysis of Starch

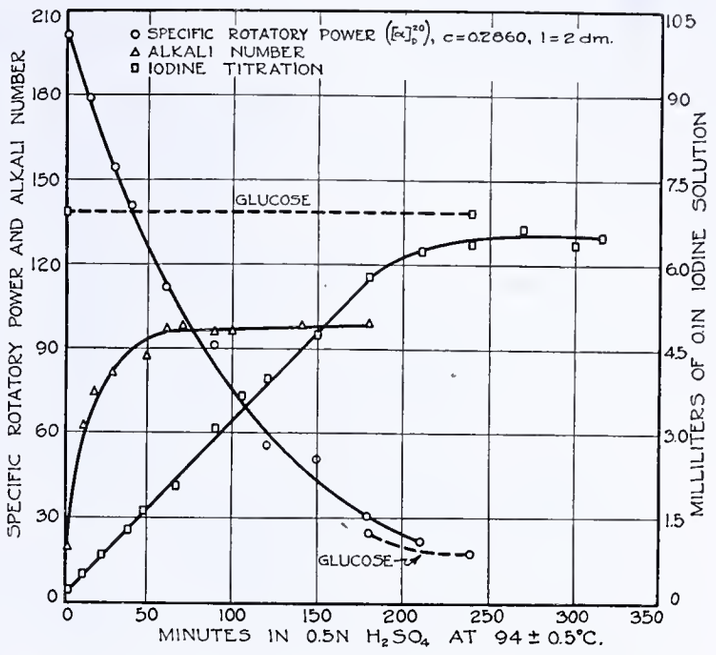
SPECIFIC ROTATORY POWER ($[\alpha]_D^{20}$), c=0.2660, l=2 dm.
ALKALI NUMBER
IODINE TITRATION
GLUCOSE
GLUCOSE
SPECIFIC ROTATORY POWER AND ALKALI NUMBER
MILLILITERS OF 0.1N IODINE SOLUTION
MINUTES IN 0.5N H_2SO_4 AT 94 ± 0.5°C.

Figure 2. Change of Specific Rotation, Alkali Number, and Iodine Titration during Hydrolysis of Starch

Procedure

Five hundred milligrams of the starch sample are weighed into a 300-ml. Erlenmeyer flask, and 25 ml. of alkaline ferricyanide reagent and 5 ml. of 0.2 per cent glucose solution are added. The flask is placed in a boiling water bath for exactly 15 minutes and is rotated while the starch is gelatinizing in order to give a uniform mixture. At the end of 15 minutes the flask is cooled under the tap to room temperature, 25 ml. of sulfuric acid solution are added, and the resulting green solution is titrated dropwise with standard ceric sulfate. The color change is from green to yellow. A blank determination is run on 5 ml. of the glucose solution. The equivalents of ceric sulfate are converted directly to milligrams of copper and reported as R_{Cu} units (mg. of copper per gram of starch).

$$\frac{\text{Ml. of ceric sulfate} \times \text{normality of ceric sulfate} \times 63.57}{\text{grams in sample}} = R_{Cu}$$

For starch products which have an R_{Cu} greater than 100 a smaller sample should be used.

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Potentiometric Determination of Mercaptans in Aqueous Alkaline Solutions

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A volumetric procedure for the quantitative determination of mercaptans in aqueous alkaline solutions is described. Mercaptans are precipitated with standard silver nitrate solution, and the end point of the reaction is determined potentiometrically using a silver electrode as an indicator. The method appears to be specific for mercaptans. It is free of interference by the usual impurities accompanying mercaptans in petroleum products and is accurate and sensitive.

THE large variety of sulfur compounds present in petroleum and the necessity of meeting widely different requirements of purity of petroleum products call for specific methods of treatment as well as for specific and sensitive methods of analysis. With increasing use of aqueous caustic solutions for the removal of acidic sulfur impurities (*1*, *4*, *5*, *7*), methods of analysis for sulfur compounds in such solutions have become necessary. A number of analytical methods for the determination of mercaptans in oils have been described. The method of Tamele and Ryland (*2*, *6*) combines the advantages of several older methods with the selectivity and sensitivity afforded by the potentiometric determination of the end point of the reaction. The present article describes a similar procedure suitable for determination of mercaptans in aqueous alkaline solutions.

Outline of Proposed Method

The procedure is based on the potentiometric titration of mercaptans with the silver–silver nitrate indicator system. The titration is performed in aqueous alkaline solutions to which ammonium hydroxide has been added to prevent the coprecipitation of small amounts of silver oxide with the insoluble silver mercaptides. The indicator electrode is a silver rod, either polished or coated electrolytically with a thin layer of silver sulfide; the sulfide-coated electrode is more generally applicable and gives a greater potential drop at the end point, but the polished silver electrode is satisfactory and was used, unless otherwise stated, for the titration of pure mercaptan solutions. Only a few substances are likely to interfere with the determination because of the relatively high potential values at which silver mercaptides are precipitated. Hydrogen sulfide, which is often present with the lower mercaptans in refinery scrubbing solutions, is precipitated as black silver sulfide at a considerably more negative potential than the light-colored mercaptides, and thus it cannot be readily confused with mercaptans.

Apparatus and Reagents

A suitable arrangement of apparatus is shown in Figure 1. The indicator electrode, *S*, is a silver rod, either polished or coated with silver sulfide. Any standard reference electrode may be used; a layer of mercury covered with a 0.1 *N* solution of sodium acetate, either aqueous or alcoholic, was used with satisfactory results. The electrolyte bridge was filled with the same solution of sodium acetate as used in the reference electrode. Any potentiometer with a range of 1.0 volt and moderate accuracy is adequate. Where a large number of determinations must be carried out, a continuous-reading instrument is preferable.

All reagents used were C. P. grade or better. The standard 0.1 *N* solutions of silver nitrate were prepared by exact weighing of the dry salt (Merck's reagent) and were standardized by potentiometric titration of potassium iodide (*3*). The 0.01 *N* and 0.001 *N* solutions were made from this solution as required by exact dilution. The investigation was limited to the aliphatic mercaptans including methyl, ethyl, *n*-butyl, *tert*-butyl, *n*-amyl, *n*-heptyl, and isooctyl mercaptan. The mercaptans were purchased from the Eastman Kodak Company. To obviate the difficulty of preparing standard, aqueous, caustic solutions of mercaptans which are easily oxidized by air, approximately 0.1, 0.01, and 0.001 *N* solutions were made in ethyl alcohol and the exact concentration of the solutions was determined by potentiometric titration in alcoholic sodium acetate immediately before use (*6*); samples for titration were prepared from these standard solutions by further dilution with aqueous sodium hydroxide.

Procedure

The sample of aqueous mercaptan solution is adjusted to suitable alkalinity by addition of sodium hydroxide or by dilution with water; the alkalinity should be approximately 1 *N*. Increasing the concentration above 2 to 3 *N* may actually have an adverse effect because of the "salting out" action of the stronger caustic solutions which may overshadow the slightly more favorable distribution between free mercaptan and mercaptide ions caused by the increase in alkalinity (*7*). The solution is then made approximately 0.05 *N* with respect to ammonia by the addition of strong ammonium hydroxide solution. This amount was found sufficient to prevent precipitation of dark brown silver oxide. The final volume should be about 50 to 100 ml. The silver electrode is immersed in the solution, silver nitrate (0.1, 0.01, or 0.001 *N*) is introduced while the solution is gently stirred, and potential measurements are recorded.

The polished electrode assumes a negative potential which varies with the nature of the mercaptan and the composition of the solution. For a solution 1 *N* in sodium hydroxide and 0.05 *N* in ammonium hydroxide, and using alcoholic 0.1 *N* sodium acetate–mercury reference electrode, methyl and ethyl mercaptans impart the most negative potential of those tested, about −0.6 volt; isooctyl next, approximately −0.5 volt for the same solution; and intermediate mercaptans

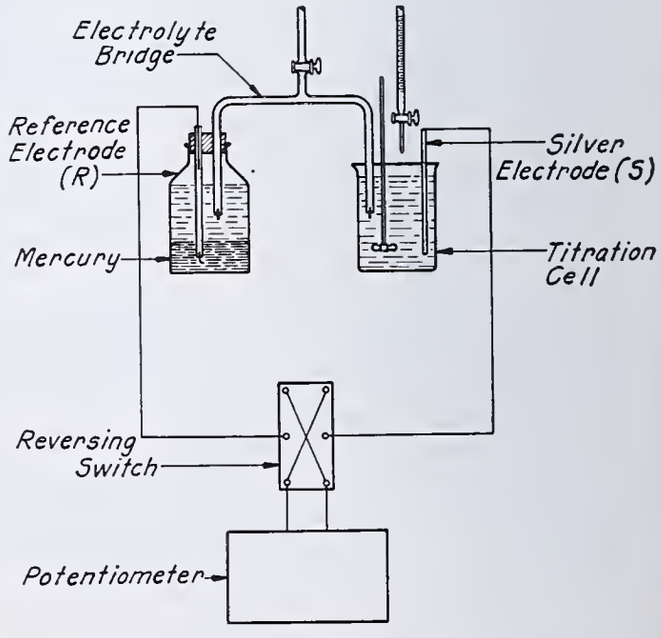

FIGURE 1. APPARATUS FOR POTENTIOMETRIC TITRATION OF MERCAPTANS

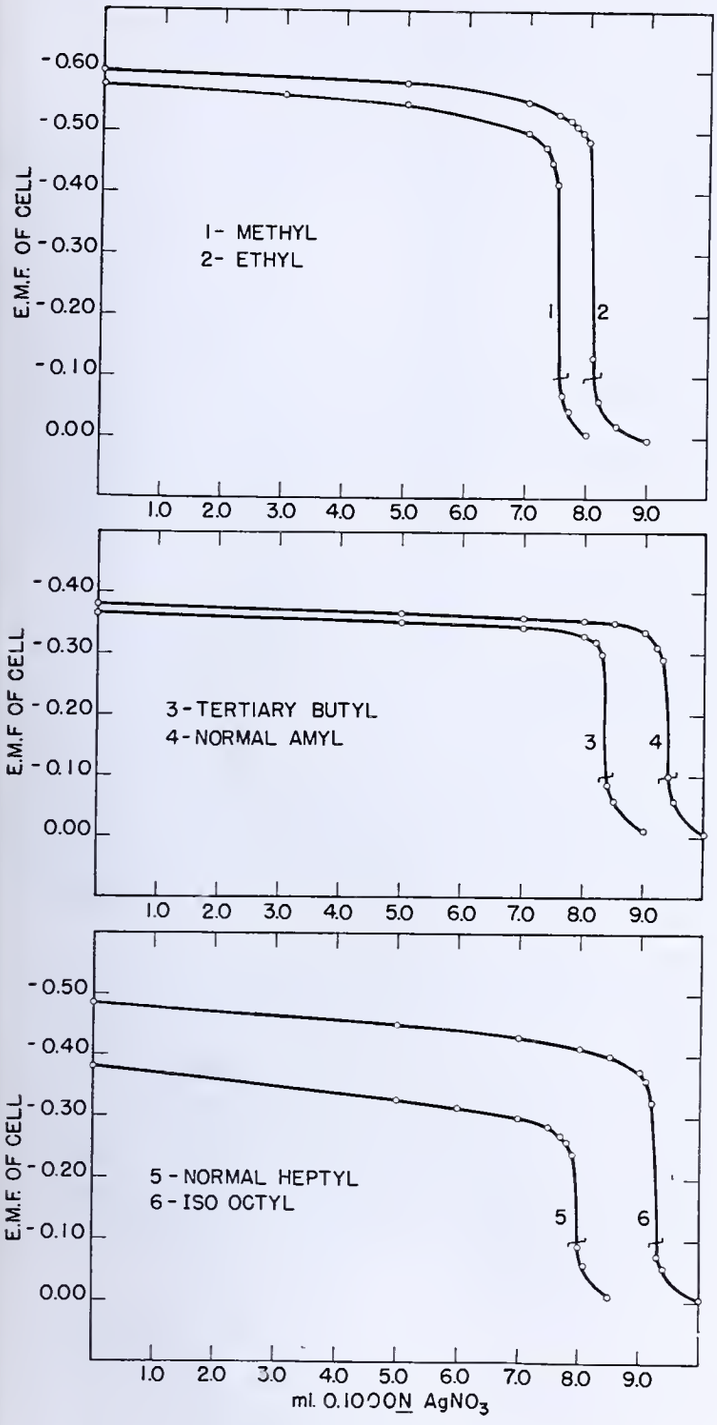

FIGURE 2. POTENTIOMETRIC TITRATION OF MERCAPTANS

Reference electrode: Hg|0.1 *N* alcoholic NaOAc
Indicator electrode: Ag|1.0 *N* NaOH, 0.05 *N* NH_4OH

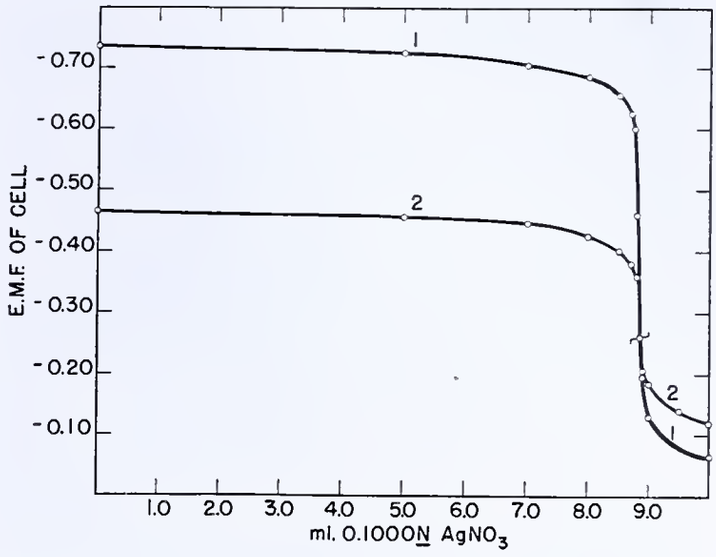

FIGURE 3. TITRATION OF *n*-BUTYL MERCAPTAN WITH POLISHED SILVER AND SULFIDE-COATED SILVER INDICATOR ELECTRODE

Reference electrode: Hg|0.1 *N* aqueous NaOAc
Indicator electrode: Ag or Ag, Ag_2S|1.0 *N* NaOH, 0.05 *N* NH_4OH
1. Sulfide-coated electrode. 2. Polished silver electrode

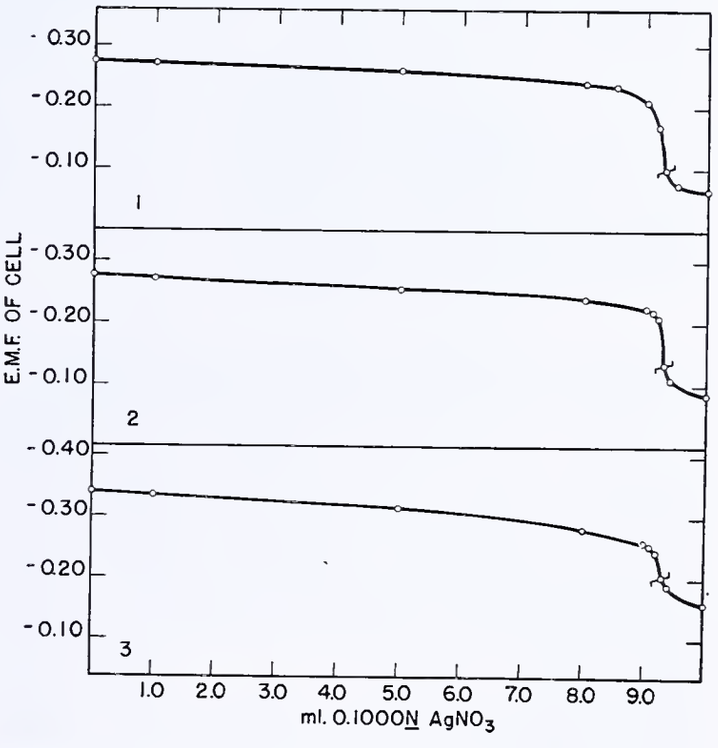

FIGURE 4. EFFECT OF CONCENTRATION OF SODIUM HYDROXIDE ON TITRATION OF *tert*-BUTYL MERCAPTAN

Indicator electrode: Polished Ag|(*x*) *N* NaOH, 0.05 *N* NH_4OH
Reference electrode: Hg|0.1 *N* alcoholic NaOAc
1. 0.1 *N* NaOH. 2. 1.0 *N* NaOH. 3. 10.0 *N* NaOH

around −0.3 to −0.4 volt (cf. Figure 2). The sulfide-coated indicator electrode assumes a more negative potential than the polished silver electrode when immersed in the same solution of mercaptan. This difference is marked in most cases; with *n*-butyl mercaptan, it amounts to approximately 0.28 volt. Thus, with the sulfide-coated electrode the break at the end point is more pronounced (Figure 3).

The end point of the titration is not taken at the inflection point of the curve but at the most positive potential value of the almost linear descending portion of the titration curve. This end point has been derived empirically from the titration of a large number of solutions of known concentration. The e. m. f. of the cell at the end point varies substantially with the composition of the solution (Figure 4). Increasing the concentration of caustic causes the initial potential of either indicator electrode to be somewhat more negative, but this advantage is more than offset by a shift to more negative values of the final potential which the electrodes assume when the mercaptan has been completely precipitated. The total change in potential is therefore less as the concentration of caustic is increased.

The more negative potential of the sulfide-coated electrode in mercaptan solutions is of distinct advantage in the presence of ions which form insoluble silver salts. Thus, the titration of mercaptans in the presence of thiosulfate with the polished silver electrode gives a barely detectable inflection

point; but with the sulfide-coated indicator electrode, the change in potential is much greater and more distinct (Figure 5).

Interfering Substances and Conditions

Because of the relatively high values of potential at which silver mercaptides are precipitated, the chances of encountering an interfering substance are small. Tamele and Ryland (*6*) demonstrated the absence of interference by a number of substances occurring in petroleum on the precipitation of silver mercaptides in alcoholic sodium acetate; a number of these substances are insoluble in water and are not likely to be encountered in aqueous alkaline solutions.

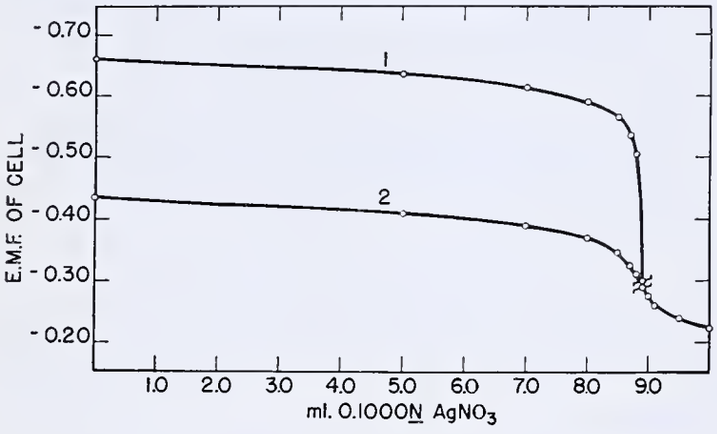

FIGURE 5. TITRATION OF *n*-BUTYL MERCAPTAN IN PRESENCE OF SODIUM THIOSULFATE

Indicator electrode: Ag or Ag, Ag_2S|1.0 *N* NaOH, 0.05*N* NH_4OH, 0.01 *N* $Na_2S_2O_3$
Reference electrode: Hg|0.1 *N* aqueous NaOAc
1. Sulfide-coated electrode. 2. Polished silver electrode

The presence of water-soluble sulfur compounds which form silver salts insoluble in ammonium hydroxide is likely to be disturbing. However, the presence of sulfide and thiosulfate ions, which are most commonly encountered in company with the mercaptans, can be readily recognized from the shape of the titration curve. Sulfide ions are precipitated at a more negative and thiosulfate ions at a more positive potential than mercaptans; and within certain limits, mercaptans can be determined quantitatively in the presence of both these substances. The analysis of mixtures of hydrogen sulfide and mercaptans will be described in a subsequent paper.

Errors may arise from various sources: oxidation of mercaptans in alkaline solutions, loss of mercaptans by evaporation, and coprecipitation of silver oxide due to lack of sufficient amount of ammonium hydroxide in the solution.

The coprecipitation of silver oxide with silver mercaptide cannot be detected from the shape of the titration curve (Figure 6), but will be apparent if the color of the silver precipitate is observed, since silver mercaptides vary in color from white for the lower mercaptans to light yellow for the higher ones, while silver oxide is dark brown. Thus coprecipitation results in a distinctly noticeable darkening of the mercaptide precipitate, and in such cases more ammonium hydroxide should be used.

In the titration of individual mercaptans, the most favorable conditions may be estimated with reasonable certainty from the data on solubility, ionization constant (*7*), volatility, and consideration of the potentials. Thus it is possible to perform a titration of methyl and ethyl mercaptans without the addition of ammonium hydroxide and reach the end point before precipitation of silver oxide begins. In general, however, this procedure is not practical, and in some cases is impossible, particularly in mixtures and when mercaptans with low initial potentials are present. The difficulty of such a procedure is illustrated by the following experiment with *n*-butyl mercaptan.

TABLE I. POTENTIOMETRIC TITRATION OF 0.00918 *M* *n*-BUTYL MERCAPTAN SOLUTION

Concentration of Mercaptan in Titration Cell, Found	Deviation	Electrolyte	pH
Mole/l.	%		
0.00795	−13.4	Aqueous buffer mixture	7.0
0.00786	−14.4	Aqueous buffer mixture	8.0
0.00795	−13.4	Aqueous buffer mixture	9.0
0.00768	−16.2	Aqueous buffer mixture	10.0
0.00814	−11.3	Aqueous buffer mixture	11.0
0.00860	− 6.4	Aqueous buffer mixture	12.0
0.00924	+ 0.7	0.1 *N* NaOH	13.0
0.00924	+ 0.7	1.0 *N* NaOH	14.0

TABLE II. POTENTIOMETRIC DETERMINATION OF MERCAPTANS

(In 0.1 *N* NaOH and 0.05 *N* NH_4OH)

Mercaptan	Mercaptan Concentration in Titration Cell, Present	Found	Deviation
	Mole/l.		%
Methyl	0.00855	0.00855	0.0
	0.001050	0.001050	0.0
	0.000095	0.000095	0.0
Ethyl	0.00805	0.00805	0.0
	0.000795	0.000795	0.0
	0.000080	0.000080	0.0
n-Butyl	0.01050	0.01050	0.0
	0.001070	0.001070	0.0
	0.000103	No end point	...
tert-Butyl	0.00945	0.00935	−1.1
	0.000930	0.000930	0.0
	0.000093	0.000093	0.0
n-Amyl	0.00935	0.00925	−1.1
	0.000935	0.000925	−1.1
	0.000094	0.000094	0.0
n-Heptyl	0.01000	0.00985	−1.5
	0.001000	0.001000	0.0
	0.000100	0.000100	0.0
Isooctyl	0.00965	0.00915	−5.2
	0.000950	0.000925	−2.6
	0.000095	0.000095	0.0

TABLE III. POTENTIOMETRIC DETERMINATION OF MERCAPTANS

(In 1.0 *N* NaOH and 0.05 *N* NH_4OH)

Mercaptan	Mercaptan Concentration in Titration Cell, Present	Found	Deviation
	Mole/l.		%
Methyl	0.00855	0.00855	0.0
	0.00105	0.00105	0.0
	0.000095	0.000095	0.0
Ethyl	0.00805	0.00805	0.0
	0.000795	0.000795	0.0
	0.000080	0.000080	0.0
n-Butyl	0.01050	0.01050	0.0
	0.00107	0.00107	0.0
	0.00010	No end point	...
tert-Butyl	0.00945	0.00935	−1.1
	0.000930	0.000930	0.0
	0.000093	0.000093	0.0
n-Amyl	0.00935	0.00935	0.0
	0.000935	0.000935	0.0
	0.000094	0.000094	0.0
n-Heptyl	0.01000	0.01000	0.0
	0.001000	0.001000	0.0
	0.000100	0.000100	0.0
Isooctyl	0.00965	0.00925	−4.1
	0.000950	0.000925	−2.6
	0.000095	0.000095	0.0

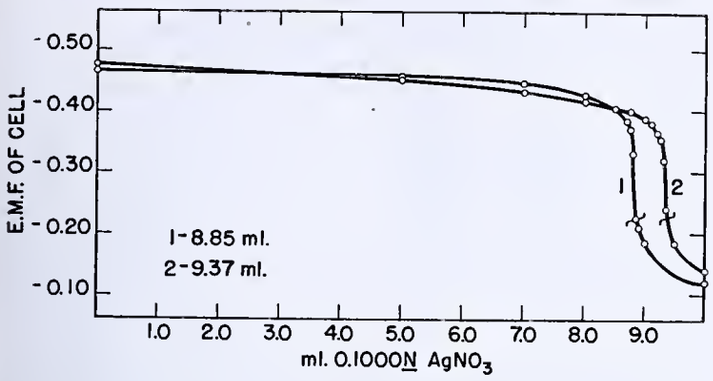

FIGURE 6. EFFECT OF AMMONIUM HYDROXIDE ON DETERMINATION OF *n*-BUTYL MERCAPTAN

Indicator electrode: Polished Ag|1.0 *N* NaOH with and without 0.05 *N* NH_4OH
Reference electrode: Hg|0.1 *N* aqueous NaOAc
1. With 0.05 *N* NH_4OH. 2. Without NH_4OH
Calculated end point = 8.85 ml.

A series of aqueous buffer mixtures (approximately 0.2 *N* salt solutions) adjusted to definite pH values in unit increments was prepared and 100 ml. of each solution were placed in the cell; to each solution 10 ml. of alcoholic 0.1010 *N* *n*-butyl mercaptan solution were added. The titrations with silver nitrate were performed without the addition of ammonium hydroxide; the results are given in Table I.

Below pH 13, serious evaporation losses occur owing to the presence of undissociated mercaptan; it was observed that the loss of mercaptan increased with time and also with the speed of stirring. As the pH of the cell electrolyte was increased from 10 to 13, the evaporation losses were materially reduced. At this alkalinity the ionization constant of 2.2×10^{-11} (*7*) indicates that approximately 99 per cent of the mercaptan is present as the sodium salt. However, slightly high results were obtained at pH 13 and 14, owing to the coprecipitation of silver oxide during the titration. It is therefore recommended that 1.0 *N* caustic be used and the coprecipitation of the oxide be prevented by the addition of a sufficient amount of ammonium hydroxide.

Mercaptans in alkaline solutions are easily oxidized by air to disulfides and other compounds. Serious errors far in excess of the inherent analytical error of the determination can easily arise from this source. It is recommended that all reasonable precautions be taken to protect the alkaline sample from needless exposure to the atmosphere and that the titration be performed without unnecessary delay.

Influence of Concentration

Three series of determinations were made in solutions of 0.1, 1.0, and 10.0 *N* sodium hydroxide. The concentration of mercaptans in the cell solution was varied from approximately 0.01 to 0.0001 *N*. The titrations were performed as follows: Mercaptan solutions in ethyl alcohol, approximately 0.1, 0.01, or 0.001 *N*, were standardized immediately before use. Ten milliliters of the solution of the desired strength were placed in the titration cell, the volume was brought to 95 ml. with the solution of sodium hydroxide, and 5 ml. of approximately 1.0 *N* ammonium hydroxide were added. (Thus the determinations were actually carried out in an aqueous 10 per cent solution of alcohol.) The results are assembled in Tables II, III, and IV.

The best results were obtained in solutions of 1.0 *N* sodium hydroxide. The small error reported in the titration of the most concentrated solution of tertiary butyl mercaptan cannot well be ascribed to the loss of mercaptan from the cell by evaporation; the solubility (0.0107 mole per liter) and the dissociation constant (0.89×10^{-11}) indicate that in solutions containing 0.01 mole per liter all of the mercaptan should be retained by 1.0 *N* sodium hydroxide (*7*). This error appears thus to be merely accidental. However, the errors observed in the titration of the more concentrated solutions of isooctyl mercaptan are attributed to the loss of mercaptan from the cell through evaporation or occlusion. Extrapolation of the data reported by Yabroff (*7*) for the solubility of mercaptans in water indicates that the solubility of isooctyl mercaptan is in the neighborhood of 10^{-5} mole per liter. Assuming further that the dissociation constant of this mercaptan is of the same order as those of the lower mercaptans (C_2 to C_7), the concentration of undissociated mercaptan in a 0.01 *N* solution of mercaptan in 1.0 *N* sodium hydroxide is of the order of 10^{-5} mole per liter. Undoubtedly the solubility of undissociated isooctyl mercaptan in 1.0 *N* sodium hydroxide is considerably less than 10^{-5} mole per liter. Thus in the more concentrated mixtures a separate phase of undissociated, undissolved isooctyl mercaptan could be expected. In the titration of the most concentrated mixture, the separation of an oily phase was actually observed. However, because of these same considerations, scrubbing solutions containing enough isooctyl mercaptan to cause these errors would not be encountered in practice. For the analysis of nonaqueous solutions containing mercaptans, it is recommended that the titrations be performed in alcoholic medium (*6*).

TABLE IV. POTENTIOMETRIC DETERMINATION OF MERCAPTANS

(In 10.0 *N* NaOH and 0.05 *N* NH_4OH)

Mercaptan	Mercaptan Concentration in Titration Cell: Present	Found	Deviation
	Mole/l.		%
Methyl	0.00855	0.00855	0.0
	0.001050	0.001050	0.0
	0.0000945	No end point	...
Ethyl	0.00805	0.00805	0.0
	0.000795	0.000795	0.0
	0.000078	No end point	...
n-Butyl	0.01050	0.01050	0.0
	0.001070	0.001080	+0.9
	0.000103	No end point	...
tert-Butyl	0.00945	0.00945	0.0
	0.000930	0.000930	0.0
	0.000093	0.000093	0.0
n-Amyl	0.00935	0.00935	0.0
	0.000935	0.000935	0.0
	0.000094	No end point	...
n-Heptyl	0.01000	0.01000	0.0
	0.001000	0.001000	0.0
	0.000100	No end point	...
Isooctyl	0.00965	0.00945	−2.1
	0.000945	0.000930	−1.6
	0.000095	No end point	...

The results obtained in 0.1 *N* sodium hydroxide solutions show considerable errors, especially in the titration of the higher mercaptans. These can likewise be attributed to evaporation and occlusion losses. Increasing the concentration of sodium hydroxide to 10 *N* reduces the sensitivity of the method at least tenfold and does not increase the accuracy of the method over that obtained in 1.0 *N* sodium hydroxide. Apparently the beneficial effect which could be expected with a shift in the equilibrium favoring the presence of a greater proportion of the isooctyl mercaptan as isooctyl mercaptide ion is offset by the salting-out effect of the stronger caustic solution.

Accuracy

The accuracy attainable by the method appears to be influenced by a number of factors, including the nature and concentration of the mercaptan, its solubility and volatility, and the oxidizability of its alkaline solution. With due precautions and under the specified conditions the accuracy appears to be about equal to the accuracy of the standardiza-

tion of the alcoholic solutions by the potentiometric method of Tamele and Ryland (*6*). Alkaline solutions of mercaptans are very readily oxidized by air. Errors greater than those inherent in the method may arise from undue exposure of samples to atmospheric oxygen. By careful manipulation as described, the errors can be kept within reasonable limits without resorting to air exclusions.

Precision

The precision of the results is difficult to estimate accurately, owing to the above-mentioned instability of dilute mercaptan solutions. Without taking any special precautions it was found that the standard deviation (root mean square deviation) as determined from fifteen titrations of a sample of *n*-butyl mercaptan with 0.01 *N* aqueous silver nitrate in 1.0 *N* sodium hydroxide and 0.05 *N* ammonium hydroxide was within 0.05 ml. It is believed that this figure is affected somewhat by oxidation of the standard mercaptan solution, as the results (Table V) show a slight trend to lower values as successive samples were withdrawn.

Table V. Precision of the Method

Sample No.	Sample *Grams*	0.01 *N* $AgNO_3$ *Ml./5 grams*	Sample No.	Sample *Grams*	0.01 *N* $AgNO_3$ *Ml./5 grams*
1	5.70	6.62	9	5.18	6.56
2	5.00	6.61	10	4.96	6.52
3	5.40	6.68	11	5.38	6.65
4	5.41	6.68	12	5.68	6.57
5	5.79	6.59	13	5.00	6.63
6	4.73	6.61	14	5.14	6.63
7	5.32	6.69	15	5.54	6.57
8	5.16	6.52			

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Sulfamic Acid Modification of the Winkler Method for Dissolved Oxygen

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ACCORDING to Gordon and Cupery (*3*, *4*) the recent development of a practical process for commercial scale manufacture of sulfamic acid (NH_2HSO_3) has aroused much interest in this chemical. It has long been known that nitrites react rapidly with sulfamic acid to yield nitrogen and sulfuric acid. A novel industrial application of this reaction is the removal of excess nitrite following diazotization—for example, in dye and lake manufacture. Sulfamic acid is two to six times more effective on a weight basis than urea, which is commonly used for such purposes. This ability to react with and destroy nitrites led to the authors' belief that sulfamic acid might be substituted for sodium azide as a preliminary treatment for removal of nitrites in the Winkler method for dissolved oxygen.

Properties of Sulfamic Acid

Dry sulfamic acid is a stable, nonhygroscopic, odorless, colorless, crystalline product. It is moderately soluble in water, its solubility varying from 14.68 grams at 0° to 47.08 grams at 80° C. per 100 grams of water. Its solubility in water is decreased by presence of sulfuric acid or sodium sulfate and becomes negligible in 70 to 100 per cent sulfuric acid. Sulfamic acid is highly ionized in aqueous solution. The strength of the acid is indicated by its reaction with basic oxides, hydroxides, and carbonates to form sulfamates. These salts are practically all soluble in water.

Sulfamic acid is essentially stable in water solution at ordinary temperature. At elevated temperatures the acid is slowly hydrolyzed to ammonium acid sulfate (*3*). Salts of sulfamic acid are stable in neutral or alkaline solution, and such solutions may be evaporated to dryness on the steam bath without hydrolysis of the amide group.

In cold solution, chlorine, bromine, and chlorates oxidize sulfamic acid to sulfuric acid. Potassium permanganate, chromic acid, and ferric chloride exert no oxidizing action. Nitric acid yields nitrous oxide and sulfuric acid. Nitrites react rapidly with sulfamic acid, liberating nitrogen and forming sulfuric acid. This reaction may be utilized for the analytical determination of nitrites or of sulfamic acid (*2*, *5*).

The properties indicate that sulfamic acid might prove a desirable agent for the destruction of nitrites in the determination of dissolved oxygen and biochemical oxygen demand. It has recently been shown that sodium azide is very satisfactory for this purpose in stream-pollution and sewage studies (*1*, *6*, *7*). Sulfamic acid is theoretically only two thirds as effective as sodium azide on a weight basis, as the following equations show:

$$NH_2SO_2OH\ (97) + HNO_2 \rightarrow H_2SO_4 + N_2 + H_2O$$

$$NaN_3\ (65) + H_2SO_4 + HNO_2 \rightarrow N_2 + H_2O + N_2O + NaHSO_4$$

However, sulfamic acid possesses the advantages of cheapness, availability, nonpoisonous nature, stability in sulfuric acid, and the formation of soluble sulfamates. Consequently, a comparative study of sulfamic acid and sodium azide for nitrite destruction in the dissolved oxygen determination was undertaken.

Experimental

A comparative study of the reaction times of 2 per cent sodium azide and 4 per cent sulfamic acid solutions showed that the reaction between sodium azide and nitrites in acid solution proceeded more rapidly than the corresponding reaction with sulfamic acid. However, the latter reaction was sufficiently rapid to justify further experimental work on the use of sulfamic acid as a nitrite-destroying reagent.

Table I. Comparative Dissolved Oxygen Data on Water, Dilute Sewage, and Treatment Plant Effluent Samples

	Dissolved Oxygen[a]		
	Azide modification	Sulfamic acid modification	Deviation
	P. p. m.	*P. p. m.*	*P. p. m.*
Water + 2 p. p. m. of nitrite N	7.74	7.84	0.10
Water + 4 p. p. m. of nitrite N	7.92	7.92	0.00
Water + 6 p. p. m. of nitrite N	7.85	7.89	0.04
10 per cent sewage	8.10	8.17	0.07
10 per cent sewage + 5 p. p. m. of nitrite nitrogen	7.96	8.04	0.08
Activated sludge effluent	8.96	8.98	0.02
Activated sludge effluent + 5 p. p. m. of nitrite nitrogen	8.78	8.88	0.10
Trickling filter effluent	7.96	8.03	0.07
Trickling filter effluent + 5 p. p. m. of nitrite nitrogen	7.84	7.86	0.02

[a] Each value is mean of three determinations.

Tables of solubility of sulfamic acid (*3*) in varying concentrations of sulfuric acid show that a 20 per cent sulfuric acid solution will dissolve approximately 5 grams of sulfamic acid per 100 ml. of solution at 30° C. This being true, it should be possible to add the sulfamic acid as a solution in dilute sulfuric acid in the preliminary treatment for nitrites. Accordingly, the sulfamic solutions used in all of the following experiments, unless otherwise stated, were prepared as follows:

Four grams of sulfamic acid are dissolved in 50 ml. of distilled water and 50 ml. of cold 40 per cent sulfuric acid are added. In this manner very little heat is evolved, thus eliminating the possibility of hydrolysis of the amino group. The solution thus prepared is slightly turbid, but this is of no consequence. One milliliter of this solution was used as preliminary treatment for destruction of nitrites in a 300-ml. dissolved-oxygen sample bottle. As applied, this quantity of sulfamic acid will destroy 19.3 p. p. m. of nitrite nitrogen and is ample for all samples likely to be encountered in water and sewage work. After addition of the reagent the bottle is stoppered and shaken and then allowed to stand for 10 minutes. From this point on, the procedure is identical with that of the azide modification.

Solutions of varying concentrations of nitrites in distilled water were prepared containing from 2 to 6 p. p. m. of nitrite nitrogen. Dissolved oxygen was then determined on this water by the azide and sulfamic acid modifications. A similar comparison was run on sewage dilutions, trickling filter effluent, and activated sludge effluent both alone and with addition of nitrites.

Enough nitrogen was liberated in the destruction of the higher concentrations of nitrites to cause the manganese hydroxide floc to rise to the top of the bottle. However, upon reshaking the bottles in the course of dissolved-oxygen determinations, the bubbles were disengaged from the precipitate, which then settled well. The results are given in Table I. The deviations in the results obtained with these modifications vary between 0.02 and 0.10 p. p. m. and are of the same order of magnitude whether or not nitrite was added to the samples. These data demonstrate the efficacy of the use of sulfamic acid in the dissolved-oxygen procedure.

A series of experiments was carried out toward the development of a shorter, simpler procedure employing sulfamic acid. It was found that the addition of sulfamic acid along with the alkaline iodide reagent (like the azide in the original Alsterberg procedure) was valueless, because the sodium sulfamate formed in alkaline solution does not remove nitrite and the sulfamic acid produced after the final acidification did not remove it in the presence of free iodine. It is possible to apply the sulfamic acid with the manganous sulfate solution and allow time for the nitrite removal reaction before the alkaline iodide reagent is added. This was tried, employing 2 ml. of a manganous sulfate solution (480 grams of manganous sulfate tetrahydrate and 40 grams of sulfamic acid per liter) in a 300-ml. sample.

The data obtained in the determination of dissolved oxygen by several procedures in the presence of nitrites and copper and nitrites and organic matter are given in Table II. They indicate that all modifications tried were satisfactory in the presence of 100 p. p. m. of copper and 14.4 p. p. m. of nitrite nitrogen. In the presence of large amounts of organic matter the nitrites were removed effectively by all modifications tried except C, employing the manganous sulfate-containing sulfamic acid. This procedure definitely fails to remove nitrites satisfactorily in the presence of peptone or glucose. Although the so-called short Winkler technique was employed on these samples, the effect of oxidation of peptone and glucose during alkalinization with the subsequent reduction in the dissolved oxygen by 0.30 to 0.41 p. p. m. from the initial values in the distilled water is indicated by all satisfactory nitrite-removing modifications.

Azide and Sulfamic Acid as Preservatives for Dissolved Oxygen Samples

In field studies of sewage-treatment plants or polluted streams, it is sometimes desirable or necessary to delay the determination of dissolved oxygen for several hours after the samples have been collected. To do this it is necessary to inhibit biochemical oxidation, besides taking precautions to prevent gain or loss of oxygen due to temperature changes. It has been shown (*6*) that the sulfuric acid–sodium azide treatment is more effective for preserving the initial dissolved oxygen values than either sulfuric acid treatment alone or the application of the complete Winkler procedure followed by the iodine titration after the interval of storage. The efficacy of the sulfamic acid treatment for dissolved-oxygen sample pres-

Table II. Comparative Dissolved Oxygen Results

(Obtained under adverse conditions with several azide and sulfamic acid modifications)

Concentration of Interfering Substances Added to Distilled Water	Initial D. O. in Distilled Water	D. O. Results Obtained with Each Modification				
		A	B	C	D	E
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
100 p. p. m. of Cu^{++} + 1.7 p. p. m. of nitrite N	7.76	7.77	7.75	7.79	7.74	7.72
100 p. p. m. of Cu^{++} + 8.4 p. p. m. of nitrite N	7.76	7.75	7.75	7.88	7.76	7.80
100 p. p. m. of Cu^{++} + 14.4 p. p. m. of nitrite N	7.15	7.14	7.12	7.25	7.24	7.15
1000 p. p. m. of glucose + 1.7 p. p. m. of nitrite N	7.79	7.52	7.32	7.80	7.51	7.44
1000 p. p. m. of glucose + 8.4 p. p. m. of nitrite N	7.79	7.61	7.42	10.65	7.52	7.41
500 p. p. m. of peptone, no nitrite	7.65	7.35	7.30	...	7.28	7.25
500 p. p. m. of peptone + 4.8 p. p. m. of nitrite N	7.65	7.33	7.27	...	7.30	7.31
500 p. p. m. of peptone + 9.6 p. p. m. of nitrite N	7.65	7.29	7.24	...	7.28	7.24
500 p. p. m. of peptone + 14.4 p. p. m. of nitrite N	7.65	7.30	7.25	...	7.35	7.28

A = Sulfuric acid–sodium azide preliminary treatment.
B = Sulfamic acid preliminary treatment.
C = Manganous sulfate–sulfamic acid preliminary treatment.
D = Alsterberg azide treatment.
E = C and D combined.

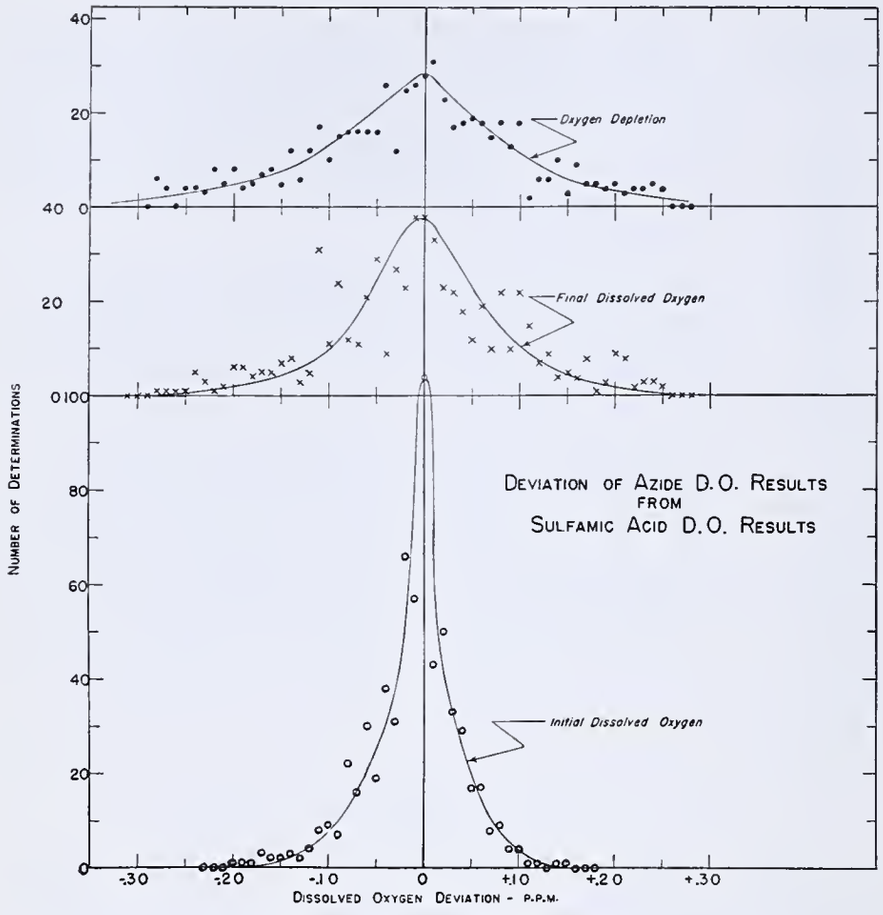

Figure 1. Dissolved Oxygen

Table III. Dissolved Oxygen Results on Preservation Tests

	25% Sewage		10% Sewage		Activated Sludge Effluent (100%)		Trickling Filter Effluent (100%)	
	Azide	Sulfamic acid	Azide	Sulfamic acid	Azide	Sulfamic acid	Azide	Sulfamic acid
Initial	8.51	8.56	8.27	8.24	7.57	7.60	7.67	7.80
2-hour interval	8.41	8.46	8.15	8.19	7.44	7.48	7.62	7.79
4-hour interval	8.10	8.25	8.02	8.15	7.33	7.32	7.38	7.56
24-hour interval	7.92	7.91	7.07	7.03	7.12	7.11	7.09	7.17
Loss of Dissolved Oxygen								
After 2 hours	0.10	0.10	0.12	0.05	0.13	0.12	0.05	0.01
After 4 hours	0.41	0.31	0.25	0.09	0.24	0.28	0.29	0.24
After 24 hours	0.59	0.65	1.20	1.21	0.45	0.49	0.58	0.63

ervation was, therefore, compared to the sulfuric acid–sodium azide treatment. The material selected for this test was a 25 per cent mixture of sewage with dilution water. This constitutes a more stringent test than is necessary for stream-pollution work.

Twenty-four bottles were put up, twelve of which were dosed with the azide reagents and twelve with the sulfamic acid reagent. Of these, six were used for initial dissolved oxygen, three with the sulfamic acid and three with the azide treatment. The remainder were allowed to stand at room temperatures and dissolved-oxygen determinations were made on these after intervals of 2, 4, and 24 hours. Similar tests were also made on trickling filter and activated sludge effluents. These results are shown in Table III, all titration figures being the average of three determinations.

Comparative B. O. D. Determinations by Azide and Sulfamic Acid Methods

In an extensive, practical, comparative study, samples from four different sources were used: the Ohio River and tributary streams in the Cincinnati area, settled sewage, an experimental trickling filter effluent, and activated sludge effluent. In all, a total of 652 duplicate samples was analyzed by the two procedures and the mean results obtained are shown in Table IV.

The individual differences between the initial dissolved-oxygen results obtained by these two modifications upon all the samples were tabulated and a frequency curve for these data is plotted in Figure 1. Positive differences signify that the azide value is greater, and negative differences that the sulfamic acid result is greater. Similar plots showing the frequency distribution of the differences in the final dissolved-oxygen results and the oxygen-depletion results are shown on higher levels in the same figure. All these curves indicate practically normal probability distribution. There may be a slight tendency for a larger frequency of negative differences in the initial dissolved-oxygen data, indicating slightly higher results for the sulfamic acid modification. This tendency is not apparent in the final dissolved-oxygen data.

The standard deviations obtained in each of the above sample groups and for all of the samples combined are given in Table V and are in general in very close agreement. There is, however, a slightly greater variation between the initial and final dissolved-oxygen deviations and between the initial dissolved-oxygen and oxygen-depletion deviations than between the final dissolved-oxygen and oxygen-depletion deviations. This is probably due to the added factor of bacterial action in oxygen consumption during the 5-day incubation period.

The sulfamic acid method is not recommended when the sample contains interfering substances such as suspensions of river mud, quantities of ferrous and ferric iron (50 p. p. m. or less of ferric iron do not interfere), sulfite wastes, or other oxidizing and reducing agents.

Summary

A modified Winkler procedure, employing a solution of 4 per cent sulfamic acid in 20 per cent sulfuric acid as a pre-

Table IV. Mean Comparative Dissolved Oxygen and Oxygen Depletion Data

(Obtained with azide and sulfamic acid modifications of Winkler method)

Samples	No. of Samples Compared	Dissolved Oxygen—Initial		Dissolved Oxygen—Final		Oxygen Depletion in 5 Days[a]	
		Azide modification	Sulfamic acid modification	Azide modification	Sulfamic acid modification	Azide modification	Sulfamic acid modification
		P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
Ohio River and tributaries	322	8.80	8.80	6.53	6.54	2.25	2.26
Raw sewage dilutions	119	8.36	8.37	5.52	5.51	2.84	2.87
Activated sludge effluent dilutions	90	8.11	8.11	6.41	6.41	1.71	1.72
Trickling filter effluent dilutions	121	8.27	8.28	5.50	5.45	2.78	2.81

[a] Values for each method are multiplied by a dilution factor to obtain 5-day B. O. D.

Table V. Comparison of Standard Deviations of Results Obtained by Azide and Sulfamic Acid Modifications of Winkler Method

Samples	No. of Samples in Series	Standard Deviation: Initial dissolved oxygen	Final dissolved oxygen	Oxygen depletion
		P. p. m.	*P. p. m.*	*P. p. m.*
Ohio River and tributaries	322	0.070	0.12	0.14
Raw sewage dilutions	119	0.073	0.18	0.19
Trickling filter effluent dilutions	121	0.052	0.15	0.16
Activated sludge effluent dilutions	90	0.047	0.15	0.16
Combined	652	0.065	0.14	0.16

liminary treatment for the removal of nitrites in the dissolved oxygen determination is as satisfactory as the azide modification for the determination of dissolved oxygen and biochemical oxygen demand in sewage-treatment and river-pollution studies.

The two modifications, azide and sulfamic acid, appear to have equal value in the prevention of biochemical oxidation when it is necessary to store a dissolved-oxygen sample for a short time.

Acknowledgment

The authors wish to acknowledge the assistance of Oliver R. Placak in the analytical work and R. S. Smith in preparation of Figure 1.

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Polarographic Determination of Ascorbic Acid

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SINCE polarographic analysis is essentially based on current voltage curves and ascorbic acid is a reducible electrolyte, this method seems suitable for vitamin C determinations. The determination is not hindered by the presence of pigments, it is specific, and it is sensitive to small amounts of electrolyte. Very complete discussions have been published by Kolthoff and Lingane (*4*) and Muller (*5*).

Preliminary vitamin C determinations were made with a Fisher Elecdropode (*2*) or polarograph. To run a determination, the beaker containing the solution to be analyzed is placed under the dropping mercury electrode. About 5 mm. of pure mercury are placed in the bottom of the beaker to act as the quiet mercury electrode and the platinum anode contact is immersed. Nitrogen is then passed through the solution as a steady stream of bubbles. This is continued for 15 minutes in order to eliminate any dissolved atmospheric oxygen, which might interfere with the curve, since it is easily reduced at the dropping electrode. Just before starting the determination, the nitrogen is started flowing across the surface of the solution instead of through it. If the gas is not thus cut off, fluctuations occur in the curve. During the determination the rate of dropping of the mercury should be kept at one drop every 3 to 6 seconds, if accurate results are to be expected. Too slow dropping causes slow oscillations in the polarogram and instability in the record. Too fast dropping produces undesirable agitation and mixing effects in the surrounding electrolyte. The dropping rate can be adjusted by changing the height of the mercury reservoir attached to the electrode.

Kodicek and Wenig (*3*) suggested the use of a 0.067 *N* phosphate buffer and used the dropping mercury electrode polarized as an anode in the determination of vitamin C on the polarograph. Since 2 per cent metaphosphoric acid has been found very satisfactory as an extractant, work was started using it as the base solution in the determination and very satisfactory curves were obtained (Figure 1). One to 3 cc. of an ascorbic acid solution, which was approximately 0.001 *N*, were added to about 50 cc. of the base solution. Curves were drawn using the dropping mercury electrode both as the anode and as the cathode. No very sharp rise was noted when the electrode was polarized as the anode; however, on one set of curves there did seem to be a very definite rise at about +0.33 volt, which was in the vicinity of that noted by Kodicek and Wenig. The difference in height caused by varying concentrations was slight even on this curve. A much sharper drop occurred at −1.77 volts. This reading was constant, varying not more than ±0.05 volt for all the curves run.

Since 0.067 *N* phosphate buffer at pH 7 had been suggested, this, an acetate buffer of pH 2.2, and a potassium acid phthalate–hydrochloric acid buffer of pH 2.2 were also tried, but none gave very consistent results. The phosphate buffer gave a rise which was not typical; the acetate buffer caused too rapid dropping of the mercury as the voltage rose and the half-wave potential came at decreasing voltages with increasing ascorbic acid concentration; the potassium acid phthalate–hydrochloric acid buffer resulted in pronounced maxima and the rise caused by the buffer occurred at the same voltage as that caused by vitamin C. As this was the only base solution causing maxima, no precautions were taken for their prevention. A 5 per cent sulfuric acid solu-

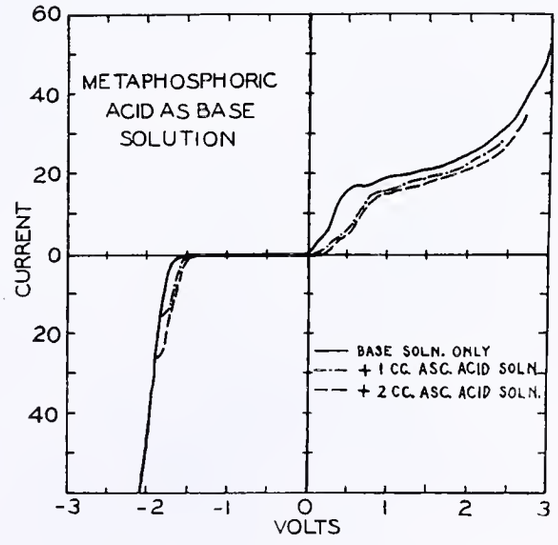

Figure 1. Polarographic Curve

tion was tried as base solution, but increased the rate of flow of the mercury drops to such an extent that no readings could be made.

Since each compound results in a characteristic rise at a specific voltage with the use of the proper base solution, the interference of such substances as cystine and glutathione would be eliminated. The acid base solution used for this ascorbic acid determination inhibits the production of current rises by these compounds. They are determined polarographically in ammoniacal solutions of cobaltous chloride in ammonium chloride (*1*).

Since this work was only preliminary, no quantitative determinations of ascorbic acid were run. However, results obtained indicated that this method could be adapted for accurate quantitative analysis by comparing the curves obtained when an unknown solution was used with calibration curves.

Acknowledgment

The author is indebted to C. G. King of the University of Pittsburgh for assistance and guidance during the investigation and to the Buhl Foundation for a research grant which made the investigation possible.

The investigation was carried out on an Elecdropode kindly supplied by the Fisher Scientific Company.

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A Simplified, Water-Jacketed, Fraction Receiver

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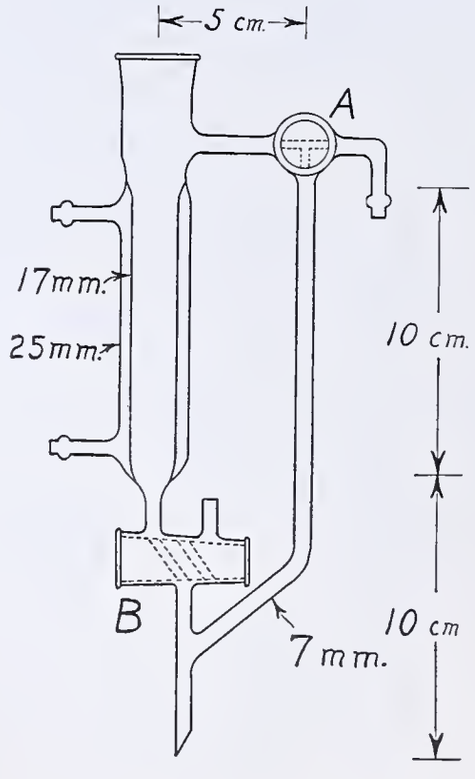
With the development of precision fractionating columns for use under reduced pressure has come a need for a receiver which will permit an indefinite number of fractions to be obtained without disturbing the pressure within the still. The older types of vacuum receivers, such as the Bruhl receiver, and the many varieties of multiple rotating receivers (*2*) are inadequate since the number of fractions which may be obtained with them is strictly limited.

Several variations of the Thorne (*4*) distillation triangle are described in Morton (*2*), some of them very satisfactory. Cloke (*1*) has recently proposed a water-jacketed modification, and Noonan (*3*) has designed a vacuum receiver in which the usual four two-way stopcocks have been replaced by a single multiple-way stopcock. The greatest disadvantage of these receivers is that they are expensive, owing to the large number or special nature of the stopcocks required, or that they require considerable glass-blowing skill to make.



The accompanying figure shows a fraction receiver which the authors developed in the organic preparations laboratory at the University of Notre Dame.

It combines the advantages of the earlier types with greater simplicity of manufacture and operation, and reduced cost. It can be easily made by an amateur glass-blower. Only two standard 2-mm. bore stopcocks are required: a three-way T stopcock (Corning No. 7420) and a three-way parallel stopcock (Corning No. 7380). The cost is considerably less than that of four two-way stopcocks. The dimensions given are those which have been found most satisfactory with the ordinary laboratory stills, but a larger or smaller reservoir may be made for special work.

The operation of the apparatus is simple. The sample is transferred from the reservoir of the fraction receiver to the receiver through stopcock *B*. *B* is closed and *A* is given a half turn, so that the receiver is disconnected from the pump but the still remains under vacuum. Air is admitted to the receiver through *B*. After the receiver containing the fraction has been removed and a fresh receiver attached, *B* is closed and *A* is turned clockwise a quarter turn, so that the still is temporarily disconnected from the pump while the new receiver is being evacuated. When the correct pressure has been established in the receiver, *A* is turned clockwise again a quarter turn, so that the still and the receiver are both connected to the vacuum line as shown.

Ground-glass joints may be used to connect the fraction receiver to the column and to the receiver, but this raises the cost without materially adding to the usefulness of the apparatus. A double tube may be used to connect the fraction receiver to the receiver to avoid any difficulty with viscous liquids rising in the equalizing arm, but for the most part this refinement has not been found necessary.

Acknowledgment

The authors wish to thank Kenneth N. Campbell for his suggestions during the course of this work.

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Determination of Peroxide Values for Rancidity in Fish Oils

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Customary peroxide methods for determining rancidity of fish oils are entirely empirical, only a portion of the peroxide reacting with the iodide to liberate iodine. The extent of the reaction depends upon the experimental conditions of the procedure used, such as peroxide concentration present, reaction time, etc. Moreover, with highly unsaturated oils, much of the liberated iodine may be absorbed by the oil.

Some of the factors controlling the reaction have been studied, and it is shown how the empirical nature of this determination can be decreased, and the reaction made more nearly quantitative and less dependent upon experimental conditions by increasing the acidity of the reaction mixture through the addition of strong mineral acids. Several other modifications in the conventional procedures are also suggested to improve precision.

PEROXIDE numbers have been widely used as an index of rancidity of oils. A number of different procedures are available for making such determinations, but results by the different methods do not always show good agreement, and in using a given method, results are often not reliable. At least with fish oils, precision is often poor, end points are vague, and correlation between peroxide number and organoleptic rancidity is uncertain.

Since fish oils are among the most unsaturated of edible oils, rancidity is of considerable importance, and the Fish and Wildlife Service has been concerned with the problem of determining it. A considerable amount of data has been obtained as to the limitations of this method for fish oils and the purpose of this report is to discuss these data.

The peroxide test suffers certain fundamental limitations which cannot be improved upon. The peroxide compounds, being intermediate and not in themselves responsible for the rancid flavors or odors, can be proportional to rancidity only so long as they are formed from the fresh oil at a greater rate than they are decomposed to the rancid products.

Thus, in the reaction: fresh oil $\xrightarrow{A}$ peroxide $\xrightarrow{B}$ rancid oil, so long as the rate of reaction A exceeds that of reaction B, the peroxide value will increase with increasing rancidity and a certain measure of its development will be obtained. If, however, the rate of reaction B should exceed that of reaction A, then the peroxide value will decrease with increasing rancidity of the oil. Accordingly, factors which affect these two rates are of importance in determining the value of the peroxide test as a measure of rancidity. One such factor is the temperature of storage of the oil. The temperature coefficient of reaction B can be considered to be much greater than that of reaction A, so that with increasing storage temperatures, peroxide numbers become less and less a reliable measure of rancidity.

Another case of the failure of peroxide values to correlate with rancidity is illustrated by the result obtained for the oil in salt fish (*4*). Such fish are normally stored at room temperature and the oil after several months' storage becomes extremely rancid. The peroxide values, however, do not give any measure of such rancidity. They show a slight rise followed by a drop to zero.

In using the peroxide test under a new set of conditions it is always first necessary to be sure a correlation is obtained with organoleptic rancidity, and unreliable results are often obtained if peroxide values obtained for an oil stored under one set of conditions are compared with those for the oil stored under some other conditions.

Errors of another type include those caused by faulty procedure. Evidence of the existence of such errors has been available for some time.

Thus, Lowen, Anderson, and Harrison (*5*) show that the peroxide value obtained depends upon the size of the oil sample used. Other errors include vague end points in some cases, poor checks on duplicate samples, and different peroxide values when different procedures are used.

Taffel and Revis (*6*) heat the oil with glacial acetic acid in the presence of nitrogen gas and solid barium or potassium iodide. They state that the greater the degree of rancidity, the more difficult it becomes to make all the peroxide react with the iodide. For very rancid oils, they recommend a longer heating period and the use of more iodide.

Wheeler (*7*) points out that in the Taffel and Revis method the oil and acetic acid form two phases, making considerable shaking necessary. He advocates the use of chloroform to form a homogeneous mixture when the iodide is added as a saturated aqueous solution. Under these conditions, he believes it unnecessary to heat the reaction mixture or to use an inert atmosphere.

Lea (*3*) proposes a method midway between that of Taffel and Revis and that of Wheeler. He uses chloroform in order to keep the oil in solution but adds the iodide as solid potassium iodide and heats the reaction mixture in the presence of nitrogen. French, Olcott, and Mattill (*1*) describe an essentially identical method.

In an effort to determine the most suitable procedure for fish oils, some of the factors involved have been studied rather comprehensively. Most attention has been paid to the Wheeler method with some comparative tests by the Lea method.

Unfortunately, it was necessary to discontinue the investigation before all the factors involved could be thoroughly studied, and consequently the methods described below are still subject to considerable improvement. However, data have been obtained which may be of interest to other workers and some definite improvements in existing procedure have been made.

In the following experiments fish oils have been used, largely salmon body oils. In most instances, an extremely rancid oil was employed since, according to Taffel and Revis (*6*), such rancid oils give the most difficulty in obtaining a quantitative reaction of peroxide with iodide.

Table I shows the effect of the sample size on the peroxide value. The Wheeler method shows a somewhat greater effect due to this cause than the Lea method. The former method gives peroxide values varying as much as 300 per cent when the sample size increases from 0.1 to 5.0 grams.

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Table I. Effect of Sample Size on Peroxide Value

Oil Sample	Peroxide Value[a] Lea Method Oil 1	Wheeler Method Oil 1	Wheeler Method Oil 2
Grams			
5.0	..	6.4	6.7
2.5	7.9	6.9	7.6
1.0	8.7	8.7	9.7
0.50	8.8	9.0	11.0
0.25	9.6	9.6	13.1
0.10	12.0	13.3	21.8
0.050	[b]	[b]	23.3

[a] Milliequivalents of peroxide per kg. of oil.
[b] End point extremely vague.

Effect of Acidity

In the determination of hydrogen peroxide a fairly high acidity is recommended (*2*). In the ordinary peroxide number determination in an oil, acetic acid, a fairly weak acid, is used. Moreover, since a nonaqueous medium is used the acidity may be much less than in aqueous medium.

Table II gives the results obtained when various strong acids are present in the solvent: 1 *N* sulfuric acid gives the highest peroxide number and also the nearest to identical results with 1- and 0.1-gram samples of oil, but it gives a very high blank; 0.1 *N* hydrochloric acid gives results next to those with 1 *N* sulfuric acid in regard to good checks with varying sample size, and fairly high peroxide numbers, and a low blank is obtained; moreover, a sharper end point is attained. Accordingly, considerable attention was given to the use of hydrochloric acid in the solvent.

Peroxide numbers were run with hydrochloric acid in the solvent in varying concentrations and using 1- and 0.1-gram samples of oil, as follows:

Concentration of Hydrochloric Acid in Acetic Acid, *N*	Peroxide Value with 1 Gram of Oil / Peroxide Value with 0.1 Gram of Oil
0	0.60
0.5	0.73
0.1	0.89
0.05	0.98
0.02	1.05

Results indicate that closest checks are obtained for the two sizes of sample when a normality of about 0.05 *N* is used. It is believed that higher concentrations cannot advantageously be used on account of the necessity of adding considerable water to the solvent. HCl = 37% gas + 63% water.

The effect of the presence of water in the solvent on the peroxide value when 1- and 0.1-gram samples of oil were used is shown by the following:

Water Added, Ml.	Peroxide Value 1 gram oil	Peroxide Value 0.1 gram oil	Peroxide Value with 1 Gram of Oil / Peroxide Value with 0.1 Gram of Oil
0	53.2	53.5	0.995
0.5	52.8	60.5	0.875
1.5	48.4	...	...
4.0	30.1	52.0	0.58

50 ml. of solvent used in each case

A number of experiments were run with hydrochloric acid solvent under different conditions. The results indicate that one of the most important factors is the oxidation of the oil by air. Not only must the air in the space above the reaction mixture be removed, but that dissolved in the solvent as well. This is best accomplished by saturation of the reagent with carbon dioxide or other inert gas. The oxidation of the oil by air occurs in the regular Wheeler method as well as in the modified procedure. Sometimes this effect is masked by a compensation of errors, the tendency toward high results through oxidation being balanced by incomplete reaction between the peroxide and iodide. When hydrochloric acid is present, not only is the reaction between peroxide and iodide accelerated but also that between any oxygen present and the oil and iodide. Hence, in the presence of strong acid, exclusion of air is of special importance.

Other conclusions drawn from these experiments include:

1. Upon standing exposed to air the peroxide value for both the Wheeler and hydrochloric acid methods increases indefinitely; if air is excluded the values tend to approach a maximum value, but only after prolonged standing.
2. In the presence of air, light accelerates the reaction.
3. Decreasing the ratio of chloroform to acetic acid gives increasing peroxide values but at the expense of precision and of accuracy with different sample sizes.
4. Standing of the oil with the solvent before adding the iodide tends to give low peroxide values, and this effect is more pronounced when using an inferior grade of acetic acid.

Table II. Effect of Acidity of Solvent on Peroxide Value

Added Acid and Its Concentration in Acetic Acid	Size of Blank, Ml. 0.005 *N* Thiosulfate	Peroxide Value with 1 Gram of Oil[a]	Peroxide Value 1 Gram of Oil / Peroxide Value 0.1 Gram of Oil
1 *N* sulfuric acid[b]	7.0	102	1.01
0.1 *N* hydrochloric acid	0.95	84	0.91
1 *N* phosphoric acid	1.3	80.5	0.87
1 *N* hydrochloric acid	1.3	90.5	0.80
0.1 *N* sulfuric acid	1.6	79	0.75
0.1 *N* trichloro-acetic acid	0.6	70	0.72
None	0.7	60.5	0.70
0.1 *N* phosphoric acid	0.6	70	0.60

[a] Milliequivalents of peroxide per kg. of oil.
[b] Very poor end point obtained.

Hydrochloric Acid Procedure

The oil is dissolved in 20 ml. of chloroform or carbon tetrachloride in a 500-ml. Erlenmeyer flask. A vigorous stream of carbon dioxide is passed through and then 30 ml. of a good grade of acetic acid containing 4 ml. of concentrated hydrochloric acid per liter are added. After about 1 minute the stream of carbon dioxide is diminished to about one bubble per second and 1 ml. of saturated potassium iodide is added. After exactly 5 minutes, 100 ml. of water (250 ml. if a dark-colored oil has been used) are added to the flask, and the iodine is titrated with dilute thiosulfate solution, adding starch and shaking vigorously near the end point.

Sulfuric Acid Method

Two disadvantages are presented by the use of sulfuric acid. A marked purple color develops which obscures the end point. The exact nature of the reaction is uncertain but it is related to the vitamin A content of the oil. An old, crude test for vitamin A was to treat the oil in chloroform and acetic acid with sulfuric acid, the color developed being proportional to the vitamin A content of the oil. A second disadvantage involves the high blanks obtained. It was found that both these disadvantages could be minimized by cooling the reagents in an ice bath before adding the iodide and keeping the flask so submerged during the reaction. Under these conditions little or no color developed to interfere with the end point and the size of the blank was greatly reduced.

Table III gives results obtained by the 1 *N* sulfuric acid method using an ice bath. For comparative purposes, the results obtained by the Wheeler method but using an ice bath and a carbon dioxide atmosphere are given. Assuming the value obtained by the sulfuric acid method after 3 hours to be correct (reaction 100 per cent complete), then the reaction in the regular Wheeler method is only 65 per cent complete and by the Lea method, only 72 per cent complete.

A more detailed study of the effect of sulfuric acid concentration on peroxide values and size of blanks is shown in Figure 1. The optimum concentration of sulfuric acid in

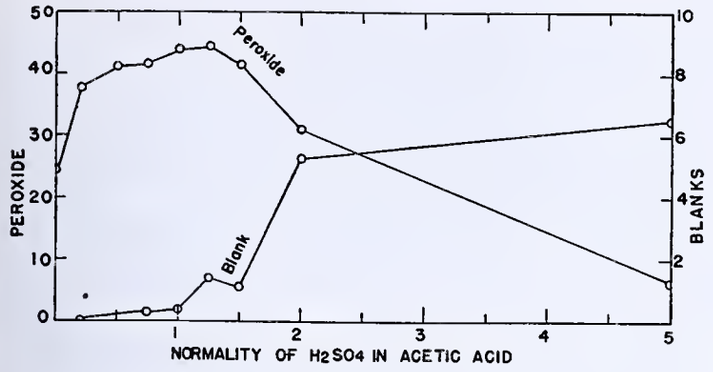

FIGURE 1

acetic acid appears to be about 1.25 *N*. Higher or lower concentrations give lower peroxide values. The size of the blank increases with sulfuric acid concentration, a marked rise occurring above about 0.6 *N*. Accordingly, a sulfuric acid concentration of 0.6 *N* in the acetic acid is suggested as most suitable, since in spite of somewhat higher results with 1.25 *N* acid, the lower blank at 0.6 *N* makes this concentration more suitable. Several other factors in connection with the use of 0.6 *N* sulfuric acid were considered and the following conclusions were drawn from the experiments made:

1. Light has no influence on the reaction in absence of air.
2. Oil must not be allowed to stand with reagents before adding iodide or low results will be obtained.
3. 1.0 ml. of saturated potassium iodide per 50 ml. of solvent gives best results.

TABLE III. PEROXIDE VALUES

(Obtained by Wheeler and sulfuric acid methods with varying reaction times)

Reaction Time	Peroxide Value[a] Wheeler method[b]	Sulfuric acid method
Min.		
1	32.2	89
5	50	101
30	63	104
Hours		
3	78	106
15	93	[c]
49	104	[c]
Regular Wheeler method	69	
Lea method	77	

[a] Milliequivalents of peroxide per kg. of oil.
[b] Modified by use of carbon dioxide atmosphere and ice bath.
[c] Blanks were higher than determination.

Sulfuric Acid Procedure

REAGENTS. Solvent. Five parts by volume of concentrated sulfuric acid are added slowly with shaking to an ice-cold mixture of 300 parts by volume of glacial acetic acid (Baker's C. P. analyzed) and 200 parts by volume of carbon tetrachloride (technical grade). The solvent is kept in a refrigerator at 0° C. until ready for use and should be prepared fresh daily.

Saturated aqueous potassium iodide (U. S. P. XI), containing 0.3 per cent sodium carbonate.

Sodium thiosulfate 0.1 *N* containing 0.01 per cent sodium carbonate.

Potassium dichromate 0.1000 *N* for standardizing sodium thiosulfate.

Before using, the sodium thiosulfate solution is diluted 20 times, and 0.01 per cent sodium carbonate is added. This dilute solution is stable for not more than 1 week.

Carbon dioxide or nitrogen in cylinder with needle valve.

Starch, 0.2 per cent, in water containing 0.001 per cent mercuric iodide.

PROCEDURE. Dissolve the oil in carbon tetrachloride which has been saturated with carbon dioxide by bubbling through a vigorous stream of the gas for 1 minute. The concentration of oil in the carbon tetrachloride should be about 5 grams made to 25 ml. in a volumetric flask.

Place 50 ml. of the cold solvent in a 500-ml. Erlenmeyer and immerse in an ice bath until the temperature is 1° C. or less. Saturate the potassium iodide solution, some distilled water, the solvent, and the starch solution with the inert gas.

Add 1 ml. of saturated potassium iodide to the flask and immediately add exactly 2 ml. of the oil in carbon tetrachloride. Allow a very slow stream of carbon dioxide to pass through the flask which still remains in the ice bath. After exactly 5 minutes add 250 ml. of water saturated with the gas, and titrate at once with 0.005 *N* thiosulfate, adding the starch near the end point. By using all reagents saturated with inert gas, no trouble is encountered with a reappearing blue starch color as is otherwise the case. The end point remains colorless for at least several hours. Run a blank in which 2 ml. of carbon tetrachloride are added in place of the oil solution.

In using this procedure great attention to detail is required in order to obtain satisfactory results. High or irregular blanks are sometimes obtained, due to a number of causes such as failure to cool the reaction mixture completely, failure to remove air completely, or use of acetic acid of inferior grade. Even when following the procedure carefully, unusually high blanks are sometimes obtained. The method is unquestionably cumbersome and for most purposes it is doubtful whether the advantages outweigh the disadvantages.

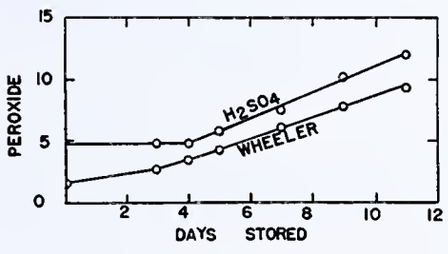

FIGURE 2

Figure 2 shows peroxide values for an oil during rancidity development determined by the sulfuric acid method and by the Wheeler method. The former method gives a sharper break in the curve at the end of the induction period. For the Wheeler method, in the early stages, very little air oxidation occurs, so that the lack of completion of the reaction tends to give low results. As the end of the induction period is reached, air oxidation counterbalances lack of completion of the reaction, so that higher results are obtained. This gives a greater slope to the curve during the induction period and makes an estimation of the end of the induction period difficult.

Other Procedures

Some experiments were run whereby the ordinary methods were drastically altered. These include substitution of other oxidation reduction systems for iodide-iodine, and use of other solvents. As shown in Table IV, most of these attempts were unsuccessful. The use of acetone in place of chloroform–acetic acid as a solvent for the oil gave some promise. The iodide was introduced as calcium iodide or as potassium iodide in methyl alcohol. As long as no water was present, the acetone did not react with the iodine, and the latter could be estimated colorimetrically by carrying out the reaction in Nessler tubes and comparing with suitable standards. The method was not worked out in detail, but might prove successful.

End Points and Precision

In titrating the iodine after the addition of water, an emulsion of some of the oil in the water layer usually is formed which is very slow to break. When a colored oil such as salmon is used, the color of the water emulsion is often of a gray-blue hue which makes the starch end point very difficult to distinguish, and either long periods of waiting for the emulsion to break are required between additions of the thiosulfate near the end point, or one must be satisfied with very poor precision. This difficulty can be minimized by the use of a large ratio of water added to solvent originally present. Using 50 ml. of solvent, about 250 ml. of water should be added if emulsion formation is to be held at a

Table IV. Results Obtained by Various Procedures

Method	Remarks
Substitution of $SnCl_2$ for KI	Reaction of Sn^{++} and peroxide too slow
Substitution of Fe^{++} for KI	Blanks too high owing to presence of traces of Fe^{+++}; water of crystallization present in some pure ferrous salts interferes
Use of excess of thiosulfate in reaction mixture to remove iodine as fast as it is liberated and prevent its absorption by oil	Thiosulfate does not titrate quantitatively to tetrathionate when present in excess in acid media; hence poor precision obtained
Use of molybdates or tungstates to catalyze peroxide iodide reaction (*2*)	Tungstate has little or no effect. Molybdate accelerates reaction but gives poor precision. Solubility of each extremely low in reaction mixture
Substitution of acetone for chloroform–acetic acid solvent. Addition of iodide in methyl alcohol or as calcium iodide in acetone	Acetone reacts slowly with iodine when water added during titration
Acetone method as above, but iodine concentration determined colorimetrically without adding water	Method gives some promise. Blanks are hard to correct for; colored oils interfere

minimum. Addition of such a large amount of water causes sufficient dilution to make the starch iodine end point less sharp, especially for oils having low peroxide values, but this effect is small compared to the difficulty obtained with the dark emulsions formed with highly colored oils.

A further improvement can be attained by adding 0.1 *N* hydrochloric acid at the end of the reaction in cases where strong acid is not already present in the reaction mixture. The presence of strong acid not only minimizes emulsion formation but also ensures the quantitative oxidation of the thiosulfate to tetrathionate rather than to sulfate (*2*) which might occur in the weak acid solution of extremely dilute iodine present (as low as 0.00001 *N* iodine). By using these simple precautions much better precision is obtained.

If difficulty is still encountered, as with some intensely colored oils, the end point can be determined electrometrically by immersing a platinum wire in the solution and connecting through a calomel half-cell to a potentiometer. The following readings were obtained with a saturated calomel half-cell, using an oil which was not highly colored in order to be able to compare the starch end point directly with the electrometric method:

0.005 *N* Thiosulfate Added *Ml.*	Starch Color	E. M. F. *Volt*
0.00	Deep blue	0.230
13.5	Medium blue	0.230
13.85	Light blue	0.230
13.91	Very light blue	0.229
13.96	Practically colorless	0.201
14.03	Colorless	0.200
14.90	Colorless	0.190

A small but decided decrease in e. m. f. is observed at the end point. In the titration of a highly colored oil, enough thiosulfate is first added until the starch color begins to fade and then readings are taken after the addition of each drop. Equilibrium is rapidly obtained and the titration can usually be completed within 5 minutes. Ordinarily, however, it is not necessary to resort to the electrometric method.

Reagents

It is of special importance to use a good grade of acetic acid. Some acetic acid, even though meeting A. C. S. specifications, gives high and irregular blanks, especially when used with the hydrochloric or sulfuric acid reagents. Other lots of acetic acid give negative blanks—i. e., they react with the liberated iodine. Each new lot of acetic acid should be tested to see if it liberates iodine from iodide under the conditions of the reaction or reacts with iodine, and if excessively high blanks are found, another supply of acetic acid should be obtained.

The chloroform seems to give no blanks whatever; carbon tetrachloride, even of a technical grade, is also suitable, and its use as an economy measure is advisable if many tests are being run.

A. C. P. or U. S. P. potassium iodide gives satisfactory results.

Stability of Reagents

The potassium iodide solution, especially in the presence of light, is oxidized rapidly to iodine. Addition of a few tenths per cent of sodium carbonate prevents such oxidation and allows the solution to be kept indefinitely.

Blank tests were made on the acetic acid–chloroform solvent with and without hydrochloric and sulfuric acid after varying storage periods. The blanks for the sulfuric acid reagent showed a rapid increase on standing, so that this reagent should be prepared daily. The other reagents showed no increase in the blank after one month, and hence can be kept for at least this long.

Some tests were made as to the stability of the dilute thiosulfate (0.005 *N*) used for the titration. The concentration changed about +1.5 per cent in 2 weeks if 0.01 per cent sodium carbonate were added and about −4 per cent without carbonate. Use of boiled distilled water and of storage in the dark did not materially increase the stability.

Recommended Procedures

The following general precautions are recommended in running peroxide tests regardless of the exact procedure; in addition, other precautions are recommended in case of special procedures.

1. Do not allow oil to stand with solvent before adding potassium iodide.
2. A technical grade of carbon tetrachloride can be used in place of chloroform as an economy measure.
3. Use the best grade of acetic acid obtainable.
4. Run both "positive" and negative blanks, the latter with standard iodine solution.

For many routine tests the Wheeler method will give results of sufficient accuracy for the purpose. Several

Table V. Relative Advantages of Methods

Methods	Advantages	Disadvantages	Application
Wheeler (*7*)	Extreme simplicity	Lack of accuracy. Poor results when sample size varied	For routine tests where speed is more important than accuracy. Use modifications recommended
Lea (*3*) or French, Olcott, and Mattill (*1*)	Moderate accuracy and precision	Inconvenience and danger of heating reaction mixture. Results still not good when sample size is varied. Use of inert atmosphere required	Hydrochloric acid method below more convenient and at least as accurate
Hydrochloric acid method	Moderate accuracy and precision	Results only fairly good when using varying sample size. Inert atmosphere required	Use for most work where accuracy is important or where sample size must be varied
Sulfuric acid method	Great accuracy and precision if details of procedure are carefully followed. Good results with large sample size variation. Sharper break in induction curve	Procedure rather involved, great attention to detail required to avoid errors	Not recommended for ordinary tests

modifications of the original method will add somewhat to the precision obtainable without decreasing the simplicity of the method.

1. Use only 10 ml. of solvent in place of 50 ml., as recommended by Wheeler.
2. Allow reaction mixture to stand for 1 minute in the dark, rather than to swirl the flask as suggested by Wheeler.
3. Use same weight of oil for all tests.
4. Add 50 ml. of 0.1 *N* hydrochloric acid in place of water at the end of the reaction. The end point is then characterized by a more rapid break in the emulsion, giving a water-clear upper layer.

When identical sample weights cannot be used (as when using aliquot portions of a tissue extract) the Wheeler method may give highly erratic results. In such cases or where greater accuracy is required, another method should be used. While the Lea method (*3*) will often suffice, the hydrochloric acid method as described above is simpler and at least as accurate. Such a procedure eliminates the necessity of heating the reaction mixture, a dangerous process at best. The acetic acid–chloroform reagent is corrosive to the skin in the cold; when heated, especially in test tubes as recommended by Lea, there is considerable danger of foaming over and serious burns may result. The hydrochloric acid method not only eliminates this danger, but gives somewhat greater accuracy when using samples of differing weights.

When the greatest accuracy attainable is desired, the sulfuric acid method can be used, but because of the close attention to detail, occasional erratic results, and numerous cumbersome precautions, this method is not recommended for general use. It is to be hoped that further work may simplify this procedure and make it more generally applicable.

Table V summarizes the advantages and disadvantages of the various methods.

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Colorimetric Determination of Lead by Diphenylcarbazide

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ADDITIONAL experience with determination of lead by the method described by Letonoff and Reinhold (*1*) has revealed several points in the procedure where difficulties may be encountered and where certain modifications, although minor, may permit more accurate results to be obtained.

The following precautions are important in connection with the reagents: (1) Ammonium acetate, 40 per cent solution, is unstable at room temperature and should be kept in a refrigerator; (2) a 15 per cent solution of acetic acid is substituted for the 25 per cent solution used originally, to avoid the possible addition of an excess; (3) lead acetate crystals used in preparation of standards should be kept well stoppered to protect against loss of water and uptake of carbon dioxide (lead nitrate is more stable and can be substituted); (4) lead chromate and acetate solutions should be stored in bottles that have been thoroughly cleaned.

The use of sodium oxalate as an anticoagulant has been abandoned. While causing no difficulty when the quantity originally specified is employed, amounts greatly exceeding the recommended concentrations have led to unreliable results, possibly because of difficulty in maintaining the calcium and lead oxalate precipitate in uniform suspension during sampling. It has been found more satisfactory to use clotted blood. Samples of blood, 5 to 8 cc. in amount, are measured into 13 × 100 mm. Pyrex tubes and allowed to clot. The tube with blood and stopper is weighed on an analytical balance to the nearest 0.1 gram, the blood is transferred to a silica dish for ashing, and the empty tube and stopper are reweighed.

Table I shows that approximately 5-gram samples of blood can be analyzed with satisfactory accuracy. Results were low for some specimens when samples much in excess of 10 grams were employed, probably because of interfering effects of salts. Samples of between 5 and 10 grams are recommended.

The method has been applied successfully to analysis of red blood cells. Samples of optimal size are obtained by allowing 5 to 10 cc. of blood to clot. After centrifugation, serum is removed and the resulting 2- to 4-gram sample of red blood cells and fibrin is used for analysis; 5 cc. of lead-free water are added to the sample and the determination is completed as described for whole blood.

Table I. Comparison of Lead Analyses on Samples of Blood

No.	Weight of Sample *Grams*	Lead Found *Mg./100 g.*	Weight of Sample *Grams*	Lead Found *Mg./100 g.*
1	5.1	0.321	9.1	0.311
2	5.9	0.033	10.0	0.032
3	6.3	0.531	13.1	0.526
4	4.6	0.055	9.7	0.054
5	5.0	0.060	9.6	0.058
6	6.0	0.056	10.0	0.054
7	5.2	0.022	10.1	0.021
8	6.0	0.050	10.3	0.051
9	5.0	0.044	10.3	0.043
10	5.8	0.043	11.4	0.044
Av.	5.5	0.122	10.4	0.119

A slight alteration in the technique of the determination has been introduced at the stage of pH adjustment just prior to precipitation of lead and immediately following the point at which the directions read "wash dish and paper four times with 1 cc. of 0.1 *N* ammonium hydroxide". At this point the control tube is employed as a standard for adjusting the pH of the unknown solutions. The control tube is prepared as described originally but instead of 1 drop of 25 per cent acetic acid 15 per cent acetic acid solution is added dropwise to each unknown until the red-orange color of the indicator in the control tube is matched. A yellow background is helpful. In this way possible addition of an excessive amount of acetic acid is excluded. The remainder of the procedure is carried out as described in the original publication.

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The Tackmeter, an Instrument for Analyzing and Measuring Tack

Application to Printing Inks

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WHILE the study and measurement of tack are of considerable interest to many industries, they are of prime importance to the manufacturer and consumer of printing inks. Proper control and adjustment of tack must be secured if "picking" is to be avoided and satisfactory "trapping" obtained. Picking occurs when the ink is too tacky, causing the surface of the paper stock to be damaged by tearing. "Trapping" is a printer's term designating the retention of one ink by another—for instance, if a red ink is to be printed on a yellow ink, the yellow, which is printed first, must retain the red ink and pull it from the printing plate as the plate leaves the paper. When this has happened it is said that the red "is trapped by the yellow". This result is satisfactory only when the order of tackiness is correct.

Comparatively little has appeared in the literature on the subject of tack, although Bekk (*1*) has published a number of papers on printing in which tack is discussed. He defines viscosity as the resistance of an ink to compression, and tack as resistance to an oppositely directed force—i. e., against the splitting of an ink layer. Bekk states that a relationship exists between tack and viscosity, but makes no attempt at an analysis which would show the mathematical relationship. He states that as far as printing is concerned, yield value will be of no consequence, but does not explain how he arrives at that conclusion.

Reed (*2*) has developed an instrument called the "Inkometer", composed of a series of rotating cylinders that are inked and in mutual contact. The ink causes a resistance to rotation. This resistance is recorded as torque which is defined as "tack".

Neither Bekk nor Reed seems to have considered the possibility of making a critical analysis of tack to discover the exact relationship between tack and the flow-resisting properties of a material, or to ascertain whether the somewhat arbitrarily arrived at definitions of tack are correct. To make such an analysis and so discover the factors upon which tack depends was the primary object in constructing the Tackmeter. Obviously, it was not known at the start, with any degree of certainty, just what phenomena produce tack. Investigators had instinctive ideas about tack but no serious attempt was made to establish their correctness experimentally. Before constructing a device for measuring tack, a preliminary analysis had to be made to indicate what qualifications such an instrument must have, but first it was necessary to select some simple test that would not only demonstrate tack but be practically synonymous with it in the mind of the practical inkman.

Both the manufacturer and the consumer of printing inks have used for many years a simple means for estimating the tack of printing inks. This is the so-called finger tap-out test, which is executed as follows:

> The ink is first rubbed out into a sufficiently thin layer with the finger tip, either on a nonporous material like glass or on coated paper. The finger tip is next firmly pressed into the film and then drawn away suddenly. When this motion is repeated a number of times in rapid succession, the sensation of stickiness arises, caused by the pull-resistance of the ink exerted on the finger tip. The amount of this pull-resistance is adjudged to be the tack.

An examination of this test shows that the ink film has divided, one part adhering to the finger and the other part remaining on the paper or plate. In order to predict what physical properties of the ink could create pull-resistance it is necessary to know how film splitting occurs. This might happen in three ways: by tearing, by abrupt rupture, or by the process of "necking down". The first of these possibilities hardly applies to liquids or to soft plastics like printing inks. Abrupt rupture would involve the tensile strength or cohesion of the liquid to such an extent that it would require thousands of pounds of force to break the film. The ink, however, being free to follow the path of least resistance will, instead, neck down to minute cross-sectional areas where rupture takes place with relative ease. Necking down is obviously, then, the most likely method by which film division occurs.

Causes of Pull-Resistance

Knowing how the splitting of printing ink films probably takes place, the next step is to understand the underlying causes of pull-resistance. Lining up the various possibilities gives the following list: (1) adhesion, (2) cohesion, (3) surface tension, (4) viscosity, and (5) yield value.

Throughout this paper the term "viscosity" is intentionally applied to plastics as well as to true liquids. The term when used in this manner must be understood to be in reference only to the cotangent of the curve—i. e., to the force in excess of the intercept divided by the rate of flow. The reciprocal of this type of viscosity is the "mobility", a term preferred by rheologists but one that would be awkward and difficult to

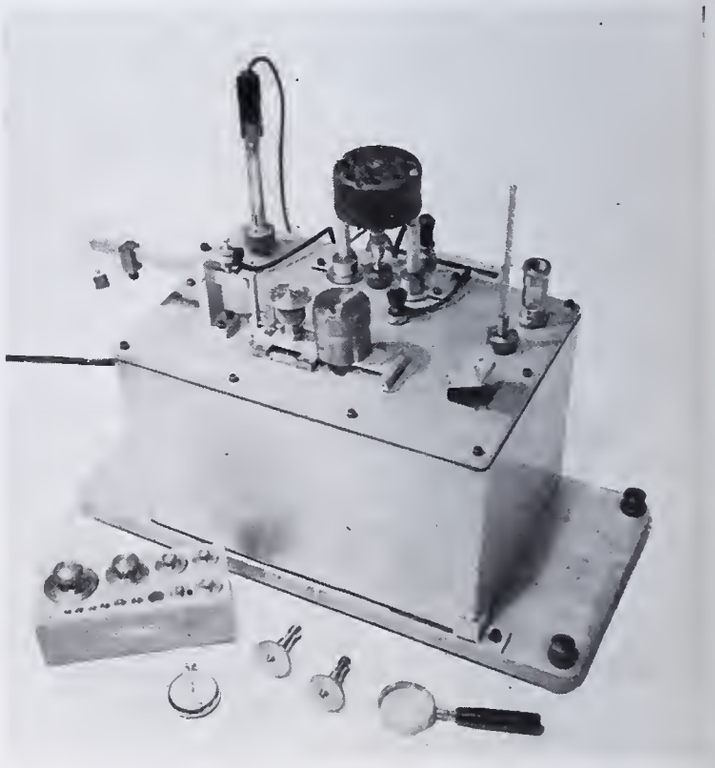

FIGURE 1. TACKMETER

introduce into the ink industry. Unfortunately there is no entirely satisfactory term analogous to viscosity that can be applied to plastics of the Bingham type.

It is difficult to imagine any other than the above five properties as being a possible cause of pull-resistance or tack. If this is so, an analysis of these properties in their relationships to the process of necking down should indicate which are the ones that a tack-measuring instrument must in some manner record.

Adhesion. This property enables the ink to adhere to the surfaces that are being separated, thus making it possible for the ink to neck down. In this connection one must be careful not to visualize the two surfaces as adhering to each other (the printing plate and the paper, or the finger tip and the paper). The ink film between the surfaces, though thin, is nevertheless of finite thickness and the two surfaces are never actually in mutual contact. The force of adhesion cannot act through the film but only at the interface between the ink and the surface involved.

Obviously, without adhesion necking down could not take place, but the magnitude of the force of adhesion, provided it is sufficient to produce complete adherence, cannot influence the magnitude of the pull-resistance. As no portion of the separating surfaces is stripped free from ink, no work is done against the force of adhesion.

Cohesion. The amount of work done against the force of cohesion at the moment of rupture must be relatively small, for the neck thins down to hairlike dimensions before breaking. It seems safe to conclude, therefore, that cohesion does not play a measurable part in creating pull-resistance. Bekk claims, however, that very thin layers of ink will rupture rather than neck down. This point will be discussed at the conclusion of the paper.

Surface Tension. When the ink film becomes divided on account of necking down, two new surfaces come into existence. Surface tension, therefore, must influence the magnitude of pull-resistance to a certain extent. Without actual Tackmeter measurements, however, the importance of this property could not be predicted. A subsequent analysis of tack measurements as shown below indicates that surface tension is of minor or negligible importance in the case of printing inks and their vehicles.

Viscosity and Yield Value. By a process of elimination it becomes apparent that pull-resistance must be accounted for mainly by the flow-resisting properties of the ink—i. e., viscosity and yield value—influenced, perhaps, to a certain extent by surface tension. The relative importance of these three properties can be determined only by experiment.

With the above in mind the Tackmeter was constructed to produce a division of the film and at the same time record the effects, if any, of surface tension, yield value, and viscosity. It was necessary that the area of contact, the film thickness, and the rate of pull (surface separation) could be varied, each one at a time, the other two remaining constant. Such an arrangement made it possible to study the effect of instrumental dimensions on the measurements.

The Tackmeter

The Tackmeter (patent applied for) is in effect a mechanical finger, paralleling in execution the finger tap-out test in its evaluation of pull-resistance, but capable of placing a numerical value on its measurements.

The instrument is shown in Figure 1 and depicted diagrammatically in Figure 2. It consists of two levers, G and J, connected by a loose-joint bar, I, the entire system being mounted on case-hardened pivots at C, D, and E. Into a threaded hole in bar G is inserted a bronze screw, A, the lower end of which functions as a finger tip. This tip is pressed into the ink at B, by placing a 500-gram weight on the bar at G. This causes the bar to rest firmly on the supports, K, K', making it possible to maintain a suitable film thickness between the lower end of A and plate L. When the 500-gram weight is removed from G, the small weight, w, at the end of lever J, exerts a pulling action on lever G, causing finger A to be pulled upward, thereby necking the ink down at B. The upward motion is stopped as soon as the right-hand side of J meets the set screw at H.

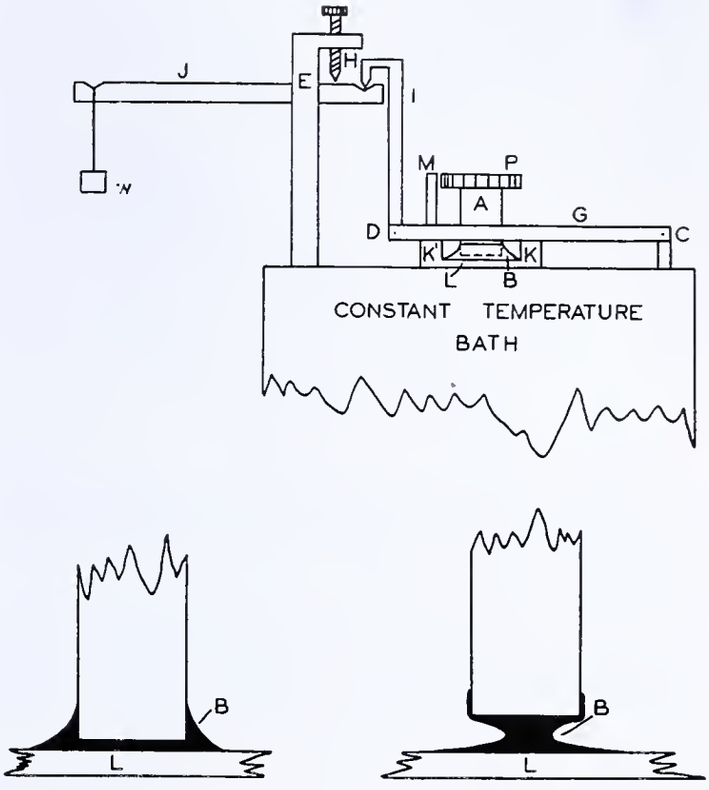

Figure 2. Diagram of Tackmeter

It is not at all necessary to use a carefully measured volume of ink. All that is required is a sufficient excess to form a reservoir of ink around the circumference of the finger tip. Because the resistance to necking down takes place under the finger tip and not around it, the exact size of the reservoir is not of serious importance. The amount of excess ink should not be so great that it touches lever arm G, nor so little that the reservoir is completely used up at the very start of the necking-down process. When the ink volume is properly adjusted variations in volume of as much as 100 per cent will not affect the tack readings.

The weight, w, will, at times, be referred to as the pull-resistance. In addition to knowing the magnitude of w it is also necessary to have the corresponding rate of break. This is obtained by taking the reciprocal of the time of break, which is measured with a stop watch, from the instant the 500-gram weight is removed until the instant the lever strikes H. It is not necessary that the necking down be carried out exactly to the point of rupture, because practically all the resistance is offered at the start and only a nonmeasurable amount exists toward the end of the operation. The entire apparatus is kept at constant temperature through an automatically controlled water bath filled so as to contact the top plate.

A measurement made like the one described gives the rate of necking down of the ink under the arbitrarily selected conditions of the experiment—i. e., with predetermined film thickness, finger-tip area, and pulling weight w. The pull-resistance is always given by the weight, w, which can be easily varied. The initial film thickness is controlled by the micrometer screw, A, by means of the graduated head, P.

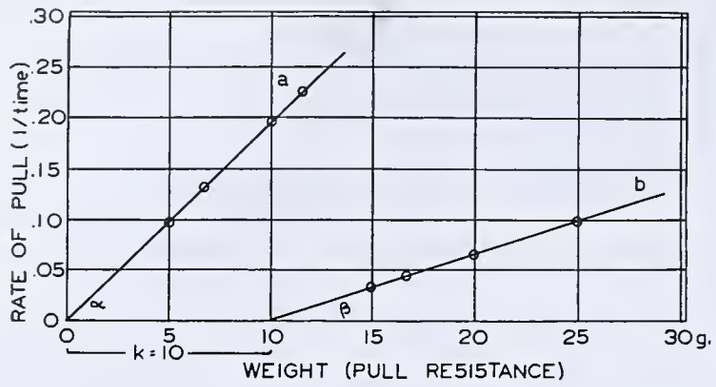

FIGURE 3. TACK CURVES

The area of the finger tip is changed by using a series of fingers with different areas. It is thus possible to vary each of the dimensional factors that influence the magnitude of the measurement.

When the time of necking down of a thixotropic material is measured on the Tackmeter, a rapid repetition of the measurement will give a series of decreasing time intervals. This is the result of thixotropic structural breakdown. The decrease is relatively large with the initial measurements but quickly diminishes to the vanishing point on repetition. The slightest loss of time encountered here before making the next time measurement will allow thixotropic build-up to increase to some former level, for at the lowest level thixotropic build-up occurs as rapidly as breakdown. As long as the material is kept in motion there is very little chance for thixotropic build-up.

TABLE I. DATA FOR TACK CURVES OF FIGURE 3

(Radius of finger tip, 0.788 cm. Film thickness, 0.01 cm., 0.004 inch)

Curve *a*		Curve *b*	
1/Time $\frac{1}{Seconds}$	Weight *Grams*	1/Time $\frac{1}{Seconds}$	Weight *Grams*
0.22	12.0	0.100	25.0
0.19	10.1	0.067	20.0
0.13	7.2	0.047	17.0
0.09	5.1	0.033	15.0
Viscosity, 20.56 poises, determined by National Bureau of Standards		Viscosity, 57.1 poises (k = 10 grams), determined with rotational viscometer	

The Law of Tack

When a given material, like an ink or a varnish, is tested on the Tackmeter with a series of different weights and these weights are plotted against their corresponding rates of break—i. e., the reciprocal of the time of break—a tack curve is produced.

From this type of curve (Figure 3 and Table I) a law can be empirically derived.

THE LAW. For Newtonian liquids the pull-resistance (tack) is directly proportional to the rate of break, $1/t$—the area of contact, πR^2, and the initial film thickness, D, remaining constant. For plastics (Bingham-bodies, using the Reiner nomenclature) the pull-resistance in excess of the intercept, k, is directly proportional to the rate of break, the other factors, as before, remaining constant.

The law states for Newtonian liquids that

$$1/t = \text{constant} \times w \quad (R \text{ and } D \text{ remaining constant})$$

and for plastics,

$$1/t = \text{constant} \times (w - k) \quad (R \text{ and } D \text{ remaining constant})$$

The constant in these equations depends on the flow-resisting factors of the material and on the instrumental dimensions, R and D. These equations can be written thus: For liquids,

$$1/t = K' K'' K''' w \quad (R \text{ and } D \text{ constant}) \qquad (1)$$

For plastics,

$$1/t = K' K'' K''' (w - k) \quad (R \text{ and } D \text{ constant}) \qquad (2)$$

where K' is a function of the flow-resisting property of the material and K'' and K''' depend on the dimensions of the instrument.

Film Thickness

The relationship of rate of break to film thickness is given for Newtonian liquids in Figure 4. If the square of the film thickness is plotted against the rate of break, w and R remaining constant, a curve will result which will not be quite linear. If to each D be added a small value, d, of such magnitude that the curve becomes rectified and passes through the origin, it will be found that d is constant for a given finger. The value for d was found to be 0.00096 cm. (0.00038 inch) for the finger used in obtaining the data for Table II. Actually the curve (Figure 4) will not reach the origin if d is constant, but end at $D = 0$. The film thickness at this point will be d, and $1/t$ will be small but finite. It is not known at present just what d represents, but it is evident that d could arise from the fact that the surfaces of the finger and the plate are not absolutely flat perfect surfaces and therefore a small space could exist between them, even at zero setting. This small space, d, plus the setting, D, on the micrometer head would be, then, the total film thickness.

The curve in Figure 4 can be written as follows: For liquids,

$$1/t = K'K''(D + d)^2 w \quad (w \text{ and } R \text{ constant}) \qquad (3)$$

where K' is a function of the flow-resisting property of the liquid and K'' is an instrumental constant.

Comparing Equation 1 with 3 shows that K''' is $(D + d)^2$. Since K''' is independent of the nature of the material being tested, its value, $(D + d)^2$, can be substituted in Equation 2. Then if $w - k$ is maintained constant and D varies, we have for plastics

$$1/t = K'K''(D + d)^2(w - k) \quad [(w - k) \text{ and } R \text{ constant}] \qquad (4)$$

Area of Finger Tip

The remaining dimensional factor that can be varied is the area of the finger tip. This is changed by using a series of fingers with different tip areas. By plotting time of break

TABLE II. DATA FOR FIGURE 4

(Radius of finger tip, 0.625 cm., 0.250 inch. d, 0.00096 cm., 0.00038 inch. w, 10.1 grams. Viscosity of oil, 20.3 poises, determined by National Bureau of Standards)

D *Inch*	$(D + d)^2$ *Inches*	1/Time *Sec.*	K (Exptl.)[a]	K (Calcd.)[b]
0.0002	0.336×10^{-6}	0.0076	0.015	0.019
0.0004	0.608	0.0151	0.032	0.034
0.0005	0.774	0.0198	0.039	0.044
0.0006	0.960	0.0241	0.048	0.054
0.001	1.90	0.052	0.106	0.108
0.002	5.66	0.164	0.331	0.321
0.003	11.4	0.323	0.652	0.647
0.004	19.2	0.560	1.10	1.09
0.005	28.9	0.830	1.68	1.64
0.006	40.7	1.15	2.35	2.31
0.007	54.5	1.53	3.15	3.09
0.008	70.2	1.98	4.08	3.98

[a] Experimental $K = \mu/wt$.
[b] Theoretically calculated $K = 4.6 \times 980\ (D + d)^2/\pi R^4$ (for c. g. s. units).

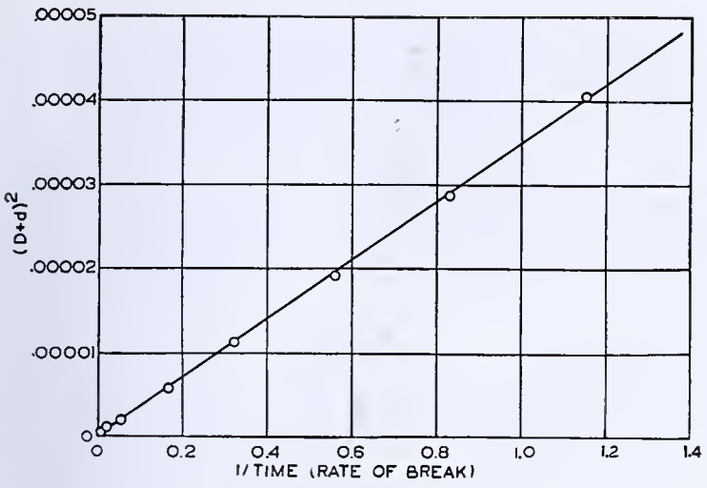

FIGURE 4. RELATIONSHIP OF RATE OF BREAK OF FILM THICKNESS

against various powers of the radii, it was found that a linear relationship results when the fourth power is used. Experimental results are shown in Figure 5. See Table III.

The curve in Figure 5 can be expressed as follows for liquids:

$$1/t = K' \left(\frac{1}{R^4}\right) K'''w \quad (D \text{ and } w \text{ constant}) \qquad (5)$$

where K' is a function of the flow-resisting property of the material, and K''' is $(D + d)^2$ as shown above.

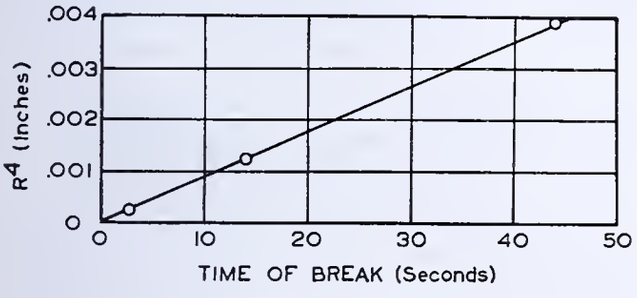

FIGURE 5. EFFECT OF AREA OF FINGER TIP

In comparing Equations 1 and 5, it will be seen in this case that K'' is $1/R^4$. Making the substitution as before gives for plastics

$$1/t = K' \left(\frac{1}{R^4}\right) K'''(w - k) \quad [D \text{ and } (w - k) \text{ constant}] \qquad (6)$$

Since the Tackmeter measures rate of flow and the force that causes it, the instrument is actually a viscometer. Therefore the curves shown in Figure 3 are rheological consistency curves. The cotangents of the angles α and β are functions of the instrumental dimensions, contact area and film thickness, and of the viscosity. The viscosity is directly proportional to the cotangent (Table IV). Yield value, therefore, must manifest itself in the intercept, k. The viscosities in Table IV were determined with a rotational viscometer.

The reason that curve b, Figure 3, is entirely linear may be that the Tackmeter uses a film thickness so thin that complete laminar flow ensues as far down as measurements can be made.

Since the cotangent is proportional to the viscosity, Equations 1 and 2 may be rewritten:

For liquids,

$$\mu = Kwt \qquad (7)$$

TABLE III. DATA FOR FIGURE 5

(Viscosity 560 poises. Heavy mineral oil. Weight, 20.1 grams. D, 0.0076 cm., 0.003 inch)

Radius, R *Inch*	R^4 *Inches*	t *Sec.*
0.250	3.906×10^{-3}	44.0
0.188	1.249	14.0
0.125	0.244	2.75

TABLE IV. VISCOSITIES

Sample	Viscosity *Poises*	Cotangent	k *Grams*	Cot/Viscosity	Material
	D = 0.003 inch, R = 0.25 inch, temperature = 29.8° C.				
1	19.8	33.8	0	1.71	Mineral oil
2	34.0	59.3	0	1.74	Varnish
3	41.5	71.5	0	1.72	Bodied linseed
4	64.0	106	0	1.66	Bodied linseed
5	73.5	122	0	1.66	Varnish
6	97.0	161	0	1.66	Varnish
7	60.0	104	4.3	1.74	Printing ink
	D = 0.004 inch, R = 0.25 inch, temperature = 29.8° C.				
8	58.0	56.0	2.8	0.97	Printing ink
9	65.0	65.8	4.8	1.01	Printing ink
	D = 0.006 inch, R = 0.25 inch, temperature = 29.8° C.				
10	70.0	38.7	4.6	0.555	Printing ink
11	86.0	48.0	7.0	0.560	Printing ink
12	114	62.0	0.7	0.550	Printing ink

For plastics,

$$\mu = K(w - k)t \qquad (8)$$

where μ is the coefficient of viscosity and K is the proportionality constant, depending only on D and R.

Combining Equations 3 and 5 gives for liquids

$$1/t = K' \frac{(D + d)^2}{R^4} (w) \qquad (8a)$$

and similarly combining 4 and 6 gives for plastics

$$1/t = K' \frac{(D + d)^2}{R^4} (w - k) \qquad (8b)$$

where K' is again some function of the flow-resisting property of the material. Equations 7 and 8 can be put into a similar form:

For liquids,

$$1/t = \left(\frac{1}{\mu}\right) Kw \qquad (9)$$

For plastics,

$$1/t = \left(\frac{1}{\mu}\right) K (w - k) \qquad (9a)$$

Since K is purely an instrumental constant, a comparison of these last four equations would indicate that $K' = 1/\mu$. When K' was given this value, however, it produced unsatisfactory results unless π was simultaneously introduced into the denominator. Such a procedure gives the final equations:

For liquids,

$$\mu = \frac{(D + d)^2 w(4.6 \times 980)t}{\pi R^4} \qquad (10)$$

and for plastics,

$$\mu = \frac{(D + d)^2 (w - k) (4.6 \times 980)t}{\pi R^4} \qquad (11)$$

where 4.6 is the lever factor and 980 is the acceleration. The actuating force, F, equals $w \times 4.6 \times 980$.

Viscosities of Newtonian liquids and plastics can be determined with the Tackmeter readily enough by means other than Equations 10 and 11, if a liquid of known viscosity is

Table V. Viscosity Measurements

Sample	Material	Tackmeter $\mu = \mu^0\left(\frac{wt}{w^0t^0}\right)$	Tackmeter $\mu = \frac{(D+d)^2Ft}{\pi R^4}$	Rotational Viscometer, μ (Poises)
D = 0.003 inch, d = 0.00038 inch, temperature = 29.8° C., R = 0.25 inch				
1	Mineral oil	20.5	20.5	(B. S. 20.56)[a]
2	Varnish	33.9	33.8	34.0
3	Bodied linseed oil	42.4	42.5	41.5
4	Bodied linseed oil	64.5	64.4	64.0
5	Varnish	74.0	73.9	73.5
6	Varnish	98.0	97.8	97.0
7	Printing ink	64.1	63.7	60.0
D = 0.004 inch, d = 0.00038 inch, R = 0.25 inch, temperature = 29.8° C.				
8	Printing ink	59.5	58.2	58.0
9	Printing ink	68.0	67.0	65.0

[a] National Bureau of Standards measurement.

used for comparison. The comparison must be made with the same film thickness and contact area for both the known and unknown materials. Then for liquids,

$$\mu = \mu^0 \frac{wt}{w^0t^0} \tag{12}$$

and for plastics,

$$\mu = \mu^0 \left[\frac{(w-k)t}{w^0t^0}\right] \tag{13}$$

where μ^0, w^0, and t^0 are the viscosity, weight, and corresponding time of break for the standard or liquid of known viscosity. Table V gives some measurements made by both methods for liquids and plastics.

Tack and Relative Tack

Tack is defined as the pull-resistance exerted by an ink film adhering between two surfaces that are separating at a definite rate and with a known area of contact and known initial film thickness. This means that tack is as much a function of the instrument that measures it as it is of the ink itself. In order to present a completely definite measurement of tack it would be necessary to give it in the form of three curves such as those in Figures 3, 4, and 5. It is very much easier and more practical to put tack on a relative basis. When tack is computed in this way the instrumental constants are practically eliminated. Relative tack is the tack of the ink divided by the tack of a standard substance such as a stable mineral oil.

Figure 6 shows two tack curves: that of the standard oil, and that of an ink.

Case I. The Tack Curve Is Linear. Let $(w_p, 1/t_p)$ be any point on the tack curve of the ink, not necessarily within the range of the instrument. This point can be the tack and rate of pull existing at the speed of the printing press. It follows from Figure 6 that

$$\frac{(w_p - k)}{1/t_p} = \frac{(w-k)}{1/t} \tag{14}$$

Then

$$w_p = \frac{(w-k)}{t_p} + k \tag{15}$$

Since w_p is the tack at speed $1/t_p$, Equation 15 can be rewritten as follows, T symbolizing tack:

$$(T)_{1/t_p} = \frac{(w-k)t}{t_p} + k \tag{16}$$

But

$$\mu = \frac{K(w-k)}{1/t}$$

and k being directly proportional to the yield value, f,

$$k = Cf \tag{17}$$

K and C are the instrumental constants depending only on the film thickness and the area of contact.

Equation 16 now can be written in terms of the rheological concepts.

$$(T)_{1/t_p} = \frac{\mu}{Kt_p} + Cf \tag{18}$$

Similarly for the standard oil

$$\frac{w^0_p}{1/t_p} = \frac{w^0}{1/t^0} \tag{19}$$

Hence,

$$(T^0)_{1/t_p} = \frac{w^0t^0}{t_p} \tag{20}$$

or

$$(T^0)_{1/t_p} = \frac{\mu^0}{Kt_p} \tag{21}$$

Dividing Equation 16 by Equation 20 gives relative tack

$$(T/T^0)_{1/tp} = \frac{(w-k)t}{w^0t^0} + \frac{kt_p}{w^0t^0} \tag{22}$$

or dividing Equation 18 by Equation 21 gives relative tack in terms of μ and f.

$$(T/T^0)_{1/t_p} = \frac{\mu}{\mu^0} + \frac{(KC)t_pf}{\mu^0} \tag{23}$$

Since tack and relative tack will be considered in this paper primarily in reference to printing press speeds, they will be expressed simply as T and T/T^0, the subscripts being omitted.

One must be careful at this point not to visualize tack and relative tack as terms synonymous with viscosity. That would be a serious misconception. Tack, as Equation 18 shows, has an infinite number of values for each ink, regardless of its viscosity, the particular value at the moment of observation depending on the speed of the press, film thickness, and area of contact. For this reason the laboratory measurement of tack is of no particular value to either the manufacturer or the consumer of inks. It therefore becomes necessary to put the measurement of tack on a relative basis and to express the tack of an ink in its relation to a substance of standard tackiness. This is accomplished in Equations 22 and 23.

The faster the press is operated the smaller t_p becomes; hence T increases with increasing press speeds. Theoretically, at infinite speeds tack becomes infinite. Relative tack, on the other hand, decreases slightly as the speed increases. At infinite speed T/T^0 reaches the finite value, μ/μ^0. This is a limiting value only and can be used only where high speeds are involved in the use of the ink.

The right-hand side of Equation 22 is composed of two terms. The first, $(w-k)t/w^0t^0$, involves viscosity but no instrumental constants; the second, kt_p/w^0t^0, depends on the yield value, on both instrumental constants K and C, and on the time of the press, t_p. The quantity t_p can be visualized as the time interval elapsing between contact and separation of the printing plate and the paper. In actual printing practice t_p is exceedingly small, so that for practical purposes the term kt_p/w^0t^0 can be omitted from Equation 22 if desired. This explains the important fact that in high-speed printing, viscosity becomes the dominant factor and the effect of yield value becomes negligible. The same inference can be drawn from Equation 16.

Case II. The Tack Curve Is Nonlinear. The principal cause of nonlinear flow curves is pseudoplasticity. Little of practical value is known about such curves. Fortunately pseudoplastic inks are the exception and not the rule. Investigators have used "one-point" methods for determining the viscosity of such materials: multiplying the cotangent (formed with the force axis and a line drawn through the point and the

origin) by the viscometer constant. That a viscosity determined in this manner complies with the definition of the coefficient of viscosity is extremely doubtful.

For the sake of argument let the coefficient of "pseudoplastic viscosity" be defined as the instrumental constant multiplied by the cotangent of the angle formed with the force axis and a line drawn tangent to the pseudoplastic curve. Also let the intercept which this line makes on the axis be defined as a function of a "pseudoplastic yield value". It is immaterial what the physical significance of these quantities is, as long as they can be measured. The tack equation for pseudoplastics then can be derived as follows:

$$\mu_p = K \frac{(w - k)}{1/t}$$

where μ_p is the coefficient of pseudoplastic viscosity.

Then

$$w = T = \frac{\mu_p}{Kt} + k$$

and

$$T/T^0 = \frac{\mu_p}{\mu^0} + \frac{Kkt}{\mu^0}$$

where K is the instrumental constant already determined and μ_p is the pseudoplastic viscosity. These equations are the same in form as those derived from linear flow curves, the only difference being the introduction of "pseudoplastic viscosity" in place of "viscosity".

Unlike viscosity, pseudoplastic viscosity is a variable, decreasing with increasing speed (*3*). While the Tackmeter can determine μ_p at low rates of flow, it cannot do so at the high speeds of the printing press. For such speeds t is small and the term Kkt/μ^0 becomes relatively unimportant. This gives, as a limiting case only, and applicable to inks on the press, the convenient formula

$$T/T^0 = \mu_p/\mu^0$$

where μ_p cannot be determined on the Tackmeter and recourse must be had to a high-speed rotational viscometer for obtaining the value in poises of the pseudoplastic viscosity.

The possible effect of surface tension, so far, has not been considered. The immediate problem is whether the surface tension of the ink or of the ink vehicle influences the value of tack or of relative tack as determined by Equations 16 and 22. Each of these equations contains but two terms; the first related to viscosity, the second, to yield value. It is conceivable that surface tension might affect either term or both. If it enters the yield value term, its effect can be considered negligible in regard to printing inks, for the yield value term itself is negligibly small at normal printing press speeds. If it affects the viscosity term to any appreciable extent, it would be manifested in the measurement of t for a given w and so produce obvious irregularities in the measurement of viscosity by Equation 10. So far nothing like this has happened. It is apparently safe, then, to conclude that in the case of printing inks and ink vehicles, the only physical properties that control tack and relative tack are viscosity and yield value.

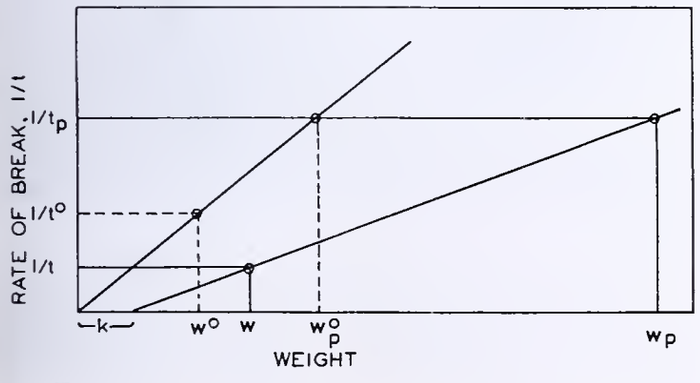

FIGURE 6. TACK CURVES OF STANDARD OIL AND INK

The relationship between tack and relative tack is such that if a series of inks gives a certain order of line-up for tack, the same order of line-up will exist for relative tack at the same speed. When two different inks give tack curves that cross, their tacks are equal at the point of crossing. The tacks above the point of crossing are in the reverse order from those below that point. These conditions also exist for relative tacks, though, of course, the complete formulas 22 and 23 must be used and not just their first terms, when making these calculations.

TABLE VI. EXPERIMENTAL TACK RESULTS FOR PRINTING INKS

(μ^0 = 1.0 poise, D = 0.025 cm., 0.010 inch)

Type of Ink		Tack, T/T^0
News ink	Black	5.0
Comic news ink	Yellow	19.0
	Blue	9.0
	Red	2.0
Rotary ink	1st down yellow	884
	2nd down red	386
	3rd down black	108
	4th down blue	54.0
Rotary ink	1st down yellow	1770
	2nd down red	464
	3rd down black	312
	4th down blue	73.0
Rotary ink	1st down yellow	550
	2nd down red	370
	3rd down blue	197
	4th down black	167
Lithographic ink (tin)	Yellow	1600
	Red	1100
	Blue	800
Photogelatin ink	Blue	2500

Picking and Trapping

Picking of the paper stock occurs when an ink is too tacky for the printing speed or for the stock used. The effect of picking is to pull off part of the surface of the paper or even to rip the stock completely. Tack measurements cannot in themselves predict whether or not an ink will pick; this can be determined only by actual trial runs. When the maximum tack that can be used successfully for a given stock and press speed is once ascertained, it follows that if this tack is exceeded, picking will occur.

The control of trapping is necessary in process printing where inks of different colors are printed successively, one on top of the other. The second down ink must be trapped by the first down; otherwise it will not print. Both inks being subjected to the same pulling force at the same moment, the one that necks down faster will be trapped by the other. Hence in a series of four-color process inks the first down must have the highest tack; the second down, a smaller tack; the third down still less, and so forth. Such an order properly spaced will ensure the optimum results in trapping. Table VI includes experimental results on the relative tacks of some process inks. For experimental convenience the standard oil used for comparison was one possessing a viscosity of 20.5 poises at 30° C. The T/T^0 values in the table were recalculated to a standard oil of 1 poise by multiplying the results by 20.5. The yield value term was not used in making the calculations. The relative tack figures therefore are those for infinite speed. On account of the fact that measurements are in "relative tack" they will differ by only a negligible amount from what they would be if calculated at the finite speed of a fast press.

Table VII. Tests of Mineral Oils

Oil	Sample No.	Film Thickness *Inch*	Viscosity *Poises*	Deviation from Average %	Maximum Deviation %
		Same sample and constant film thickness			
A	1	0.003	97.1	−0.52	
			97.2	+0.52	
			97.1	−0.52	±0.52
			97.2	+0.52	
B	1	0.004	57.1	−0.87	
			58.2	+1.05	
			58.0	+0.70	
			56.9	−1.22	±0.12
			57.5	−0.17	
			57.8	+0.35	
B	1	0.003	53.8	−1.48	
			54.5	−0.18	
			55.0	+0.74	±1.1
			55.0	+0.74	
			54.8	+0.38	
		Same sample, film thickness readjusted each time			
A	2	0.004	95.1	−0.28	
		(readjusted)	95.5	+0.14	±0.2
		(readjusted)	95.5	+0.14	
A	2	0.003	97.0	+0.34	
		(readjusted)	96.0	−0.69	±0.5
		(readjusted)	97.0	+0.34	
B	2	0.003	55.0	+0.37	
		(readjusted)	54.5	−0.55	±0.5
		(readjusted)	55.0	+0.37	
B	2	0.004	57.2	−0.52	
		(readjusted)	58.0	+0.87	±0.7
		(readjusted)	57.4	−0.17	
		Different film thickness with same sample			
A	3	0.003	96.7	+0.68	
		0.004	95.4	−0.68	±0.7
B	3	0.003	57.5	−2.3	±2.3
		0.004	54.8	+2.3	
		Different samples, constant film thickness			
B	1	0.003	54.6	−0.15	
	4	0.003	54.8	+0.22	±0.2
	5	0.003	54.6	−0.09	
B	1	0.004	57.6	−0.17	
	4	0.004	57.5	−0.35	±0.5
	5	0.004	58.0	+0.52	

Discussion

This paper describes an instrument for analyzing and measuring tack. The analysis has been attained by varying the pull-resistance, the film thickness, and the area of contact individually, studying the effect of each variation and deriving therefrom a mathematical equation relating all the factors involved. This relationship is given for liquids in Equation 10, and for plastics in Equation 11. In the form of tack and relative tack of plastics like printing inks it is given in Equations 18 and 23.

It is impossible in a preliminary report of this type, where attention has been focused mainly on printing inks, to go deeply into the possibilities and limitations of the Tackmeter. As long as the material being tested can be induced to adhere to the metal finger and plate and to neck down on pulling, there seems to be no apparent upper limit to the viscosities it can handle. The range of the largest finger used on the instrument has been, so far, from 1 poise to over 30,000,000 poises, but much research must yet be carried out before the validity of these high values can be ascertained. As far as film thickness is concerned, the Tackmeter has been operated successfully with films ranging from 0.0002 to 0.050 inch (5 to 1250 microns). Bekk has claimed that films less than 10 microns behave differently from thick ones. On the Tackmeter, oil films as thin as 5 microns and even somewhat less have been used and no evidence of a change in behavior has been detected. Materials with yield value, however, can give the appearance of a change in behavior if the force employed is either less than the yield value or so close to it that the difficulty in carrying out the measurement becomes too great to permit consistent and reliable results. This possibility is greatly enhanced by the use of very thin films.

Bekk's argument is that when he plots film thickness against force, the rate of flow being maintained unaltered, the curve produced flattens out toward the force axis as film thickness decreases. It is difficult to understand how this can be interpreted as a change in behavior, for the flattening of the curve must occur even for unchangeable materials. This can be seen from the equation of flow in capillary tubes by plotting the radius against the force, a constant volume of flow being maintained. Though Bekk does not use capillary tubes, he does employ a capillary space formed between a hemisphere and a hemispherical cup of like size and curvature. The ink or material is in this space and flows either into it or out of it, according to the way the hemisphere is moving. Bekk makes no attempt to develop the formula of his flow curve and therefore offers no proof that an unchangeable material will give any different curve from the type he gets.

If a film thickness of only several molecules is to be considered, there would be, perhaps, a different law of flow from the one given in Equations 10 and 11. Printing ink films, however, are of an entirely different order of magnitude—2 to 6 microns in process printing—and there is neither theoretical reason nor experimental proof that such films behave in any abnormal manner—i. e., differently from films, say, of 0.001 to 0.010 inch or more. As it is far more convenient to work with the larger films, the Tackmeter has been designed accordingly, though its lower limit is approximately 2.5 microns.

Determining the precision and accuracy of measurements made on printing inks is complicated by the fact that such materials are invariably thixotropic and consequently it would be difficult to decide whether structural changes in the material or shortcomings in the instrument were responsible for any unsatisfactory results. Therefore stable mineral oils have been used to investigate the duplicability of results on the Tackmeter (Table VII). Several types of duplicability have been studied: (1) where the material is not removed but remains on the instrument; (2) where different samples of the same material are checked against one another (this procedure is necessary because the quantity of sample is not determined by weighing but simply by estimation); (3) by changing the setting on the micrometer and then returning it to the original setting; and (4) by changing the setting to another film thickness and allowing it to remain there. Each measurement was made from a curve of only three points. The precision, therefore, is that obtainable for ordinary routine work and by no means represents the precision obtainable if many points are used instead of three.

The limitations of the Tackmeter are in the direction of high-speed determinations. In the case of thixotropic materials various results can be obtained, depending on the amount of thixotropic breakdown that has occurred at the time of measurement. The printing press probably destroys the thixotropic structure completely; consequently it is desirable to make tack measurements on samples that are in a similar condition of thixotropic breakdown. To approach this state on the Tackmeter it is necessary to use the following procedure.

After the ink has been placed on the instrument the lever arm, *J*, is raised at the weight end, separating the other end from the connecting bar, *I*. *I* is next moved up and down rapidly through a distance not quite great enough to break the ink completely. This operation can be carried out as rapidly as the finger tap-out test. *I* is then replaced in its notch and the measurement made in the usual manner.

The method of handling pseudoplastic inks at high speeds has been discussed above. When relative tack is required at high speeds, the viscosity term above is sufficient. This can be obtained with a properly constructed and operated high-speed rotational viscometer. Such an apparatus is useful where pseudoplasticity enters or where the ink is too heavy to undergo a satisfactory thixotropic breakdown on the Tackmeter. This conclusion might lead to the question, why, then, have a Tackmeter? The answer is obvious. Neither rotational nor capillary tube viscometers measure pull-resistance and therefore they cannot show the relation of tack and relative tack to yield value and viscosity. This relationship can be determined only by an apparatus that splits or partially splits the film. The Tackmeter does this and the relationship shown in Equations 10 and 11 is the result. When Equation 11 is written in the form of Equation 23, it can be seen that relative tack at high speeds depends practically only on viscosity. This conclusion cannot be derived from either a rotational or a capillary viscometer.

Acknowledgment

The author wishes to express his indebtedness to the Interchemical Corporation for making this investigation possible, and to Ruth N. Weltmann and William A. Elbracht for valuable cooperation in carrying out this work.

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(2) Reed, R. F., "The Inkometer", Lithographic Technical Foundation, *Tech. Bull.* 2 (Research Series 4, 1939).
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Apparatus and Methods for Precise Fractional-Distillation Analysis[1]

New Design of Adiabatic Fractionating Column and Precision-Spaced Wire Packing for Temperature Range −190° to 300° C.

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Podbielniak Centrifugal Super-Contactor Company, 8312 South Chicago Ave., Chicago, Ill.

A new column design was developed and found satisfactory over the temperature range of −190° to 300° C. with extended use of vacuum jacketing around column, flask, reflux, and all connections, and with means for compensating for residual heat leakage through vacuum jacket. New vacuum-jacketed standard-taper ground joints withstand both liquid air and 300° C. temperatures, remaining vacuum-tight and easily detachable. Flexible glass bellows are used throughout as expansion joints for differential thermal dimensional changes. The column is made of four parts to permit interchangeable use of various distilling tubes and flasks. A new form of precision-spaced wire-type packing was also developed for the fullest utilization of capillary reflux liquid films across closely spaced wire turns. This packing has tested as high as 75 plates in 35 cm. (14 inches) with the *n*-heptane and methylcyclohexane test and its low H. E. T. P. and other desirable characteristics are maintained at least up to 25-mm. diameter. Performance data are submitted for packings of various size. This type of packing is now in use in many laboratories for both low- and high-temperature fractionation.

COMPLETE and exact control of column thermal conditions and of heat input, reflux cooling, and product withdrawal is important for maximum-efficiency fractional distillation. By "maximum-efficiency fractional distillation" is meant modern high-efficiency laboratory fractionating columns and packings ranging from 50 theoretical plates upwards by test, and requiring proportionately high reflux ratios for best performance.

In another paper (*12*) illustrative data and conclusions are given on the performance of nonadiabatic fractionating columns. Actually, all laboratory fractionating columns are to a greater or lesser degree nonadiabatic, because even the best insulation is imperfect. Under certain conditions it has been experimentally proved that nonadiabatic columns test materially more plates than more nearly adiabatic columns. Under other conditions it has been possible to destroy the effectiveness of a basically 100-plate column to as low as 12 plates by supercooling or superheating it, depending on the volumes and concentrations of charge at top and bottom of the column. Present fractional distillation theory provides no adequate explanation of these findings, which have, however, been exhaustively confirmed by several years of experimental work.

In application to laboratory fractionating columns, which usually operate with a relatively large volume of charge in the kettle compared to column and condenser holdup, while super-cooling—i. e., partial condensation of ascending vapors through heat radiation from column to outside—may actually improve fractionation, although at the expense of reflux ratio, superheating may completely destroy separation no matter how effective the column may be as a contacting apparatus. In any case, for fractionation of maximum efficiency it is necessary to control the thermal surroundings of high-efficiency fractionating columns to within close limits. The insulation of such columns and the compensation of the residual heat leakage which occurs through even the best in-

[1] Patent Notice. The Heli-Grid packing and Super-Cal column described in this paper are protected by one or more of the following patents and by pending patent applications, under which the Podbielniak Centrifugal Super-Contactor Co. is exclusive licensee: U. S. Patents 1,909,315, 1,917,272, 1,935,888, 1,967,258, 2,009,814, 2,088,385, and 2,093,644; British Patent 380,220; French Patent 721,598; Canadian Patents 343,524 and 343,525; Rumanian Patent 27,009.

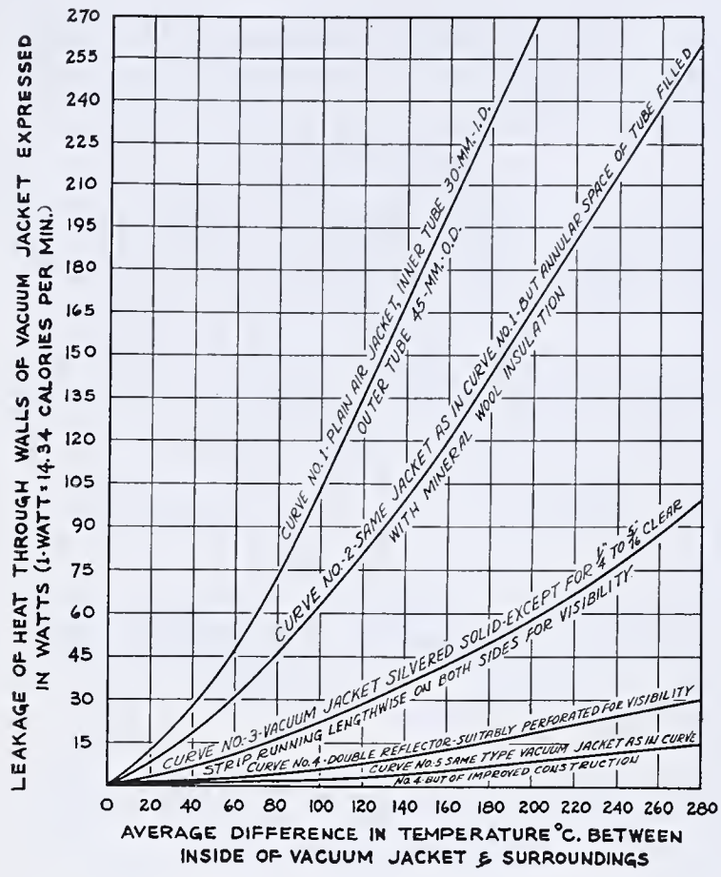
LEAKAGE OF HEAT THROUGH WALLS OF VACUUM JACKET EXPRESSED IN WATTS (1 WATT = 14.34 CALORIES PER MIN.)
270
255
240
225
210
195
180
165
150
135
120
105
90
75
60
45
30
15
CURVE NO. 1: PLAIN AIR JACKET, INNER TUBE 30-MM. I.D. OUTER TUBE 45-MM. O.D.
CURVE NO. 2: SAME JACKET AS IN CURVE NO. 1 BUT ANNULAR SPACE OF TUBE FILLED WITH MINERAL WOOL INSULATION
CURVE NO. 3: VACUUM JACKET SILVERED SOLID EXCEPT FOR 1/4" TO 5/16" CLEAR STRIP RUNNING LENGTHWISE ON BOTH SIDES FOR VISIBILITY
CURVE NO. 4: DOUBLE REFLECTOR SUITABLY PERFORATED FOR VISIBILITY
CURVE NO. 5 SAME TYPE VACUUM JACKET AS IN CURVE NO. 4 BUT OF IMPROVED CONSTRUCTION
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280
AVERAGE DIFFERENCE IN TEMPERATURE °C. BETWEEN INSIDE OF VACUUM JACKET & SURROUNDINGS

FIGURE 1. THERMAL INSULATION EFFECTIVENESS OF LARGE-BORE COLUMN VACUUM JACKETS

Computed from experimental data on 9-mm. inside diameter jackets

sulation are essential to their best performance. Careful control of both the direction and degree of departure from the adiabatic condition is highly desirable.

Another factor also enters, in that the H. E. T. P. of most high-efficiency laboratory column packings varies with the vaporization and reflux rate as much as 200 per cent or more. It should therefore be possible to operate the columns at the proper rates to secure best balance of high fractionating effectiveness and maximum useful distilling capacity. However, in most laboratory columns 75 per cent or more of the heat input to the relatively poorly insulated distilling flask is lost to the atmosphere, and it is therefore not practicable to measure vaporization by heat input. Various devices are used to measure vaporization rate, at top or bottom of column, but these are usually of only rough accuracy, not adequate for setting the column at near its optimum rate, as determined for a particular column and a particular mixture.

Finally, the measurement and control of reflux ratio for high-efficiency columns are most important in obtaining best possible separation with a column in a given time of distillation. The reflux ratio as determined by overhead measurements is equal to the over-all reflux ratio only when the column is truly adiabatic. The importance of using an adequate reflux ratio, in conjunction with an adequate number of plates, for a desired separation, is well brought out by Rose (*13*).

Problems in Design and Construction of Adiabatic Fractionating Columns

In an attempt to develop a fully adiabatic column for a wide range of temperatures, the selection of insulation is paramount. This is especially true of high-efficiency packings, which are usually of larger diameter to compensate for their reduced distilling capacity due to small clearances; there is more area to insulate and more dependence on the insulation.

Figure 1, computed from experimental data on a 9-mm. inside diameter series of air-jacketed and vacuum-jacketed columns as presented in a previous paper (*9*), represents the approximate residual heat leakages through a corresponding series of 30-mm. inside diameter air and vacuum jackets. The great superiority of the metal reflector–shielded vacuum jacket over any other form of column insulation is apparent. However, at 300° C. above room temperature the residual heat leakage is of the order of 15 watts, which would result in serious supercooling if uncompensated. As described in a previous paper (*10*), a miniature electric resistance wire heater may be used between the distilling tube or column proper and the sleeve-type vacuum jacket, with proper regulation, to compensate very closely for this residual heat leakage for all temperatures.

If the distilling tube for use within the vacuum jacket of Figure 1 were itself to be vacuum-jacketed, this additional vacuum jacket in series with the outer vacuum jacket might offset the increased heat leakage of the large-bore vacuum jacket, and make regulation of a miniature compensating heater between the two vacuum jackets far less critical. Such complication would not be warranted merely to replace proper compensatory heater regulation. However, the additional vacuum jacket is a by-product of a construction advantageous in several other respects.

A more serious problem came up in providing expansion joints for the large-bore vacuum jacket. For jacket bores up to about 9 mm. the inner tube of the vaccum jacket could be bowed deeply in the case of low-temperature fractionation, or left almost straight in the case of high-temperature fractionation, so that the resulting straightening or bowing of the inner jacket tube would take care of the inevitable differential expansion between the inner and outer vacuum-jacket tubes. However, for vacuum jackets of the order of 28-mm. bore, the inner tube is far too rigid to use the bowing expedient.

Figure 2 is a diagram of expansions and stresses in vacuum jackets under differential temperatures as indicated. The

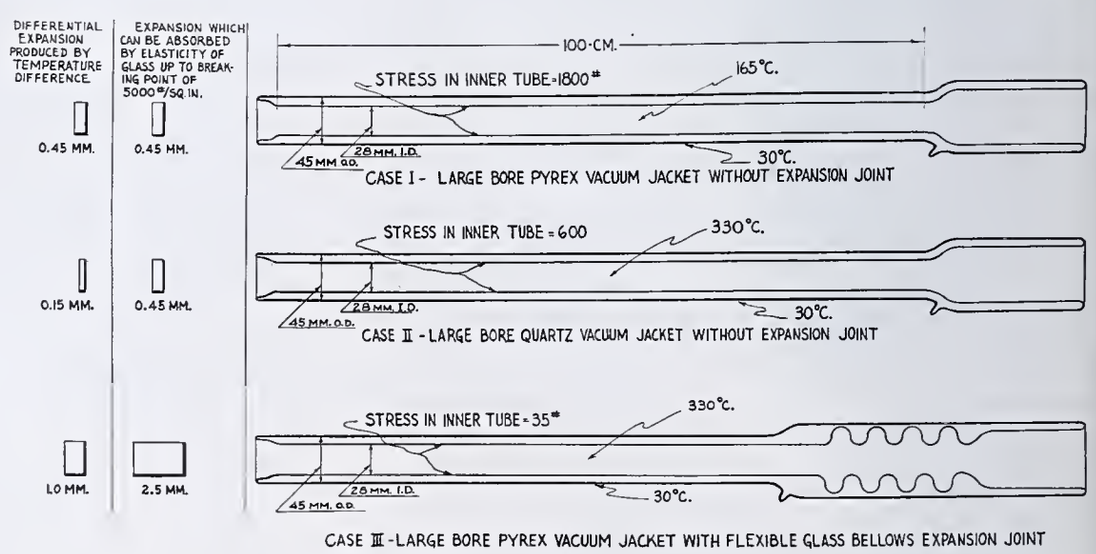
DIFFERENTIAL EXPANSION PRODUCED BY TEMPERATURE DIFFERENCE.
EXPANSION WHICH CAN BE ABSORBED BY ELASTICITY OF GLASS UP TO BREAKING POINT OF 5000 #/SQ. IN.
0.45 MM.
0.45 MM.
0.15 MM.
0.45 MM.
1.0 MM.
2.5 MM.
100 CM.
STRESS IN INNER TUBE = 1800 #
165 °C.
45 MM O.D.
28 MM. I.D.
30 °C.
CASE I - LARGE BORE PYREX VACUUM JACKET WITHOUT EXPANSION JOINT
STRESS IN INNER TUBE = 600
330 °C.
28 MM. I.D.
45 MM. O.D.
30 °C.
CASE II - LARGE BORE QUARTZ VACUUM JACKET WITHOUT EXPANSION JOINT
STRESS IN INNER TUBE = 35 #
330 °C.
28 MM. I.D.
45 MM. O.D.
30 °C.
CASE III - LARGE BORE PYREX VACUUM JACKET WITH FLEXIBLE GLASS BELLOWS EXPANSION JOINT

FIGURE 2. DIFFERENTIAL THERMAL EXPANSIONS AND RESULTING STRESSES IN VACUUM JACKETS

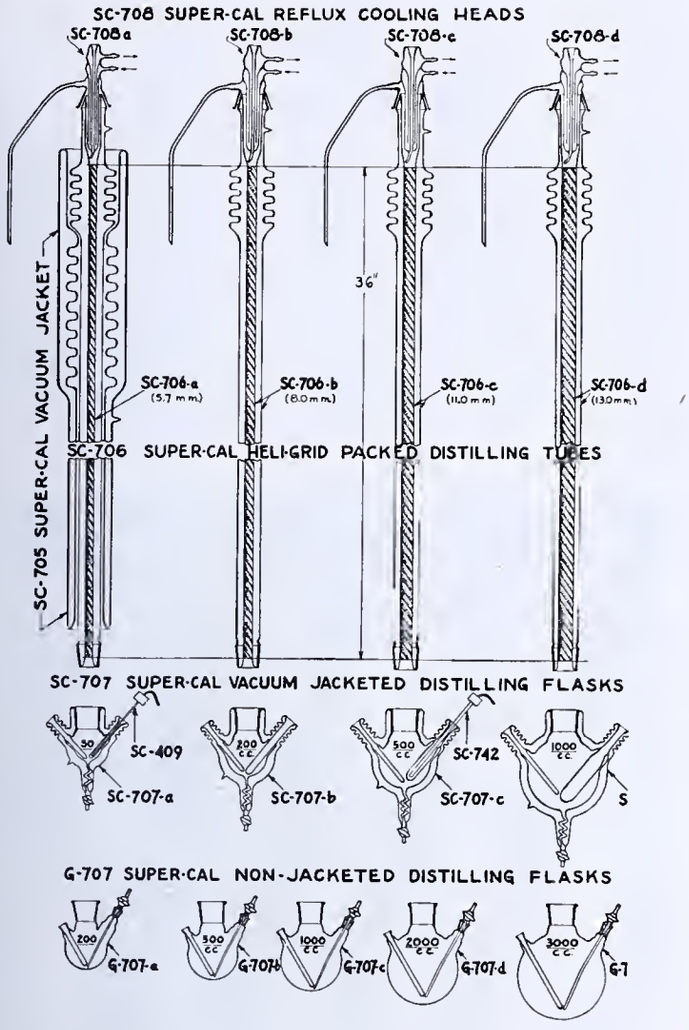

FIGURE 3. SUPER-CAL TYPE OF FULLY ADIABATIC VACUUM-JACKETED FRACTIONATING COLUMN SYSTEM

data for computation of elastic compression and stresses of glass and quartz are Morey's (7), and have been verified experimentally in this application.

The large-bore Pyrex jacket without an expansion joint cannot possibly survive about 135° C. temperature difference, as illustrated, which subjects the glass to its breaking stress, with a tremendous load on the end ring seals of the jacket. Actually, with such a severe strain, the jacket would probably break at a much lower temperature than the maximum indicated. Curiously enough, the literature contains descriptions and drawings of similar vacuum-jacketed columns without expansion joints, although intended for distillation to high temperature.

The all-quartz vacuum jacket, because of its low thermal expansion coefficient, reaches a temperature difference of 300° C. with about one third of its breaking strain (which is approximately the same as that of Pyrex). Actually, in the author's experience, such quartz vacuum jackets last indefinitely, as far as thermal strains are concerned. However, in large sizes, the cost of the quartz is prohibitive.

The third vacuum jacket in Figure 2 is Pyrex, with the incorporation of a flexible glass bellows as expansion joint in the inner tube. With proper fabricating machinery and technique, these bellows can be made amply rugged mechanically, and yet sufficiently flexible to take care of all differential expansion at 300° C. temperature difference, with more than 100 per cent safety margin and only 15.9-kg. (35-pound) strain in the inner tube. This, then, seemed the only possible and satisfactory solution of the problem of absorbing the differential expansion of a large-bore vacuum jacket, and was adopted in the columns described here.

High-Efficiency Vacuum-Jacketed Column

Figure 3 shows the Super-Cal system of interchangeable outer vacuum jacket, distilling tubes and flasks provided with vacuum jackets and ground joints, and reflux cooling heads.

Flexible glass bellows are used as expansion joints in the construction of all the column parts except the reflux cooling head, and except where inlet and outlet tubes could be bent to serve as expansion joints, as in the vacuum-jacketed distilling flasks. The flexible glass bellows expansion joint is used in the outer tube of the vacuum-jacketed distilling tubes, not in expectation of large temperature difference across these tubes in normal operation, but as an emergency provision in case of momentary large temperature differences, as when starting distillation.

The use of vacuum-jacketed standard-taper ground joints between the bottom end of distilling tubes and distilling flasks to provide a leak-tight, easily detached connection at this point has successfully overcome a very annoying difficulty in both low- and high-temperature fractionating apparatus. As rubber tubing joints or lubricated ground joints freeze at liquid air temperatures and leak, while unlubricated ground joints are not vacuum-tight at any temperature, only fused-glass joints have hitherto been satisfactory for connecting low-temperature distilling bulbs to the column. In high-temperature fractionation, corks coated with glue have been the usual but unsatisfactory solution, while ground joints cannot be relied upon at high temperatures.

With a suitable lubricant of high graphite content, the vacuum-jacketed joints between distilling tube and the vacuum-jacketed series of distilling flasks not only remain vacuum-tight at lowest obtainable temperatures, but can even be rotated at those temperatures without developing leaks. Instant interchange from one size or type of distilling bulb to another without disassembly of column has thus been made possible for low-temperature fractionation apparatus. A mercury seal, although not found necessary in practice, may be used with this type of joint to assure tightness under all vacuum conditions.

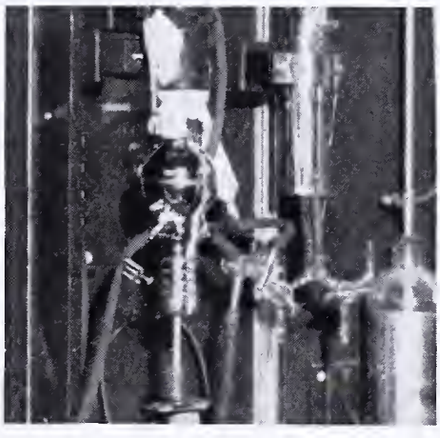

FIGURE 4. LOW-TEMPERATURE SUPER-CAL TYPE OF DISTILLING FLASK WITH BUILT-IN PRECOOLER AND HEATING WELL

The vacuum-jacketed joint with either the vacuum-jacketed or non-vacuum-jacketed series of distilling flasks has proved equally satisfactory for high-temperature fractionation. Sufficient temperature gradient exists throughout the long ground zone of the joint so that most of the lubricant remains at a low enough temperature to function satisfactorily.

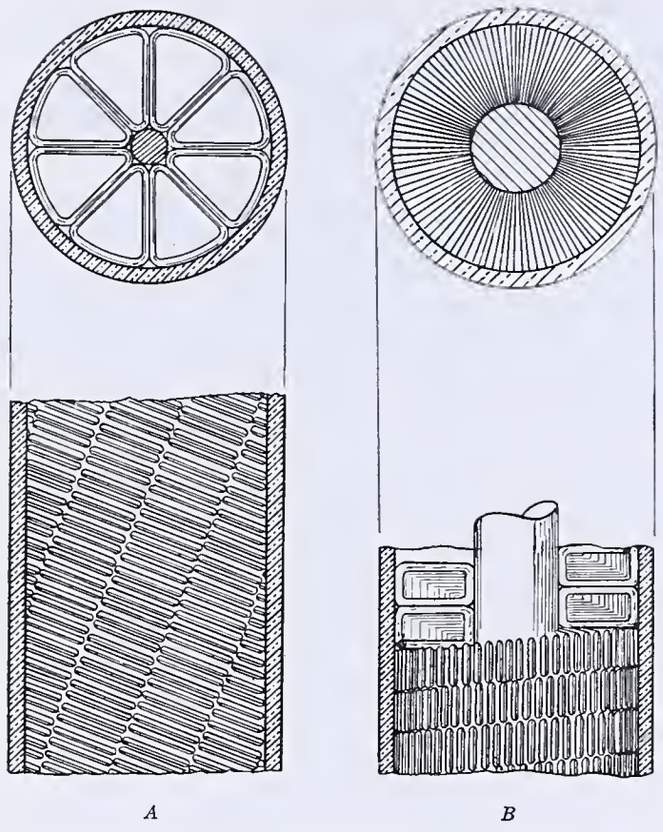

FIGURE 5. DIAGRAM OF HELI-GRID PACKINGS

A. Assembly of sector-section coils twisted around central core
B. Staircase assembly of rectangular section coils around central core

A detachable low-temperature fractionating flask must obviously carry its own vacuum jacketing, as is illustrated in Figure 4, which shows a flask of 50-cc. total capacity, with its built-in precooler element, also serving as heater well, with a cartridge-type heater, as described for the Super-Cool column (*12*).

The reflux cooling heads shown are of conventional type, for high-temperature fractionation, suitable for brine, water, or air cooling. The same remarks apply to the vacuum-jacketed upper ground joint of the distilling tubes as were made above with reference to the lower joint. The heads should be proportioned for least holdup of liquid compatible with adequate cooling surface for the capacity of distilling tube used for the lowest boiling liquids to be distilled. For low-temperature fractionation the vacuum jacket of the distilling tube terminates sufficiently below the upper end of the distilling tube to permit the use of a reflux cooling vessel and attachments as described for the Super-Cool column.

The outer tube of the vacuum-jacketed packed distilling element serves conveniently to join the upper and the lower vacuum-jacketed ground joints, without weak triple-glass seals, and to protect the weaker inner tube with its expensive packing. It also serves as a secondary (nonreflectored) insulation and as a buffer against improper regulation of the compensatory miniature resistance wire heater which is used in the annular space between the two vacuum jackets.

With the Super-Cal type of column assembly the heat lost by radiation from the vacuum-jacketed distilling flasks need not be more than a few watts, and this can be accurately allowed for, while complete compensation can be provided for in the column proper by the means described. Thus, this generalized column design provides for fully adiabatic column and still performance throughout the entire temperature range of −190° to 300° C., with no change in general design, except for adaptations of distilling flask and reflux head for low-temperature fractionation. It is actually possible, in the one column, to distill a sample ranging in composition from methane to lubricating oil, beginning with a reflux temperature of −161.4° C. at atmospheric pressure, and ending with 300° C. in the reflux at 20-mm. absolute pressure.

Construction and Performance Characteristics of Packing

The Heli-Grid high-efficiency packing was developed simultaneously with the Super-Cal column assembly, in an effort to arrive at a balanced laboratory fractionating column of maximum efficiency. Having provided the proper thermal conditions and all other operating elements, further progress was naturally in the direction of securing greatest attainable effectiveness of the vital packing element.

In the development of high-efficiency laboratory column packings, first thought was given to the possibility of improving the single wire-coil packing introduced by the author for low-temperature fractionation, because of its unusual combination of low holdup and small diameter with relatively high plates and distilling capacity. It seemed that the principle of capillary film formation between wire turns or loops spaced precisely in accordance with definite carefully worked out patterns could be extended to yield at least as low H. E. T. P.'s as packings depending on random arrangement (such as Lessing rings or single-turn wire or glass helices).

FIGURE 6. HELI-GRID PACKINGS

Starting with this basic thought, the rest of the development was entirely experimental, consisting of trying out innumerable wire patterns, sizes, spacings, loop diameters, sections, etc., in each case testing the packing variation by benzene–ethylene dichloride test mixture, and later with *n*-heptane and methylcyclohexane. Theory on surface tension phenomena was found to be of little help when dealing with liquid and vapor flows through complex wire whorls. It was necessary to concentrate on development of forming technique and tools and of special machinery for most precise and uniform execution of those packings which gave best test results,

varying details of spacing, coil diameter, section shape, etc., very slightly to detect trend of change in further test results. Only correctness of form and precise execution distinguish this type of packing from a scouring wad of tangled wire.

It was found possible to develop many forms of close-clearance wire packings which showed high efficiencies, such as the two forms illustrated in Figure 5. The packing in Figure 5, *A*, consists of coils of sectorlike section, wound around each and around a very small core wire, to yield a number of uniform nonflooding vapor passageways, "lined" perfectly with capillary liquid reflux films extending between vertically adjacent wire loops. Another form of packing (Figure 5, *B*) consists of wire cage "staircases", wound around a central solid core and around each other in concentric telescoping layers, if necessary to obtain larger diameters and capacities. Figure 6 illustrates several sizes and types of Heli-Grid packings.

This type of packing has been named the Heli-Grid because of its resemblance to helical grids or filaments. Because of its capillary spacings, it acts like a metal sponge with respect to any liquid reflux seeking to channel between packing and walls of glass tube or of the solid core, and no such channeling can occur except at actual gross overflooding. Even if the packing is tilted nearly horizontal and some liquid poured into the upper end, the liquid will not channel, but will actually run spirally up and down following the capillary wire coils. In order to assure this freedom from channeling, despite nonuniformity of bore of glass tubing used, the packing is pre-tensioned as to twist in fabrication to fill the glass tubing snugly at all points, despite irregularities. Inconel, or Nichrome, is the wire material of choice, because of its high resistance to corrosion of all types and because it can be readily cleaned with sulfuric and nitric acids without injury. Wire sizes and spacings will vary with design, but usually the wire is of the order of 0.25-mm. (0.010-inch) diameter, with spacings between wire about equal to wire diameter.

As the testing of laboratory column packings has become fairly well standardized in the technical literature, it has been possible to compare Heli-Grid packings with other high-efficiency packings as to performance characteristics (Figure 7). In representing other packings every attempt has been made to use the actual data given in papers by those introducing the packings, or to compute therefrom without error or prejudice.

The author believes that in the case of analytical batch fractionating columns, neither the height nor the diameter of the column, so long as these are not excessive, is of importance, as against the desirability of high distilling capacity and number of plates with least possible holdup of sample in column. Therefore, factor A was set up to express over-all column efficiency considering all these three important factors. Bragg (*2*) has set up a similar factor, in reciprocal form, for use in his comparison of column packing types.

The H. E. T. P. of the Heli-Grid packing, as determined by the *n*-heptane and methylcyclohexane test mixture at total reflux, is the lowest yet reported in the literature, reaching 75 plates in 35 cm. (14 inches) of packing, or about 0.5 cm. (0.2 inch) H. E. T. P. As is shown in Figure 8, these low H. E. T. P.'s have been verified on 108-cm. (36-inch) packings, up to the limit of the *n*-heptane and methylcyclohexane test.

Likewise, as regards holdup per plate and factor A, Heli-Grid packings compare very favorably with other packings, throughout most of their distilling capacity range. Like many other high-efficiency low-holdup packings, the H. E. T. P. *vs.* reflux rate curve falls off rapidly with increasing reflux rate. Conversely by operating at low rates, the Heli-Grid packing attains remarkable fractionating effectiveness without the usual falling off at low rates shown by many other packings. This behavior would seem to show complete freedom from channeling of either vapor or liquid, at the lower rates.

Strangely enough, the Heli-Grid packing requires preflooding in the manner described by Fenske and others, for attainment of highest efficiency. This was not originally appreciated in

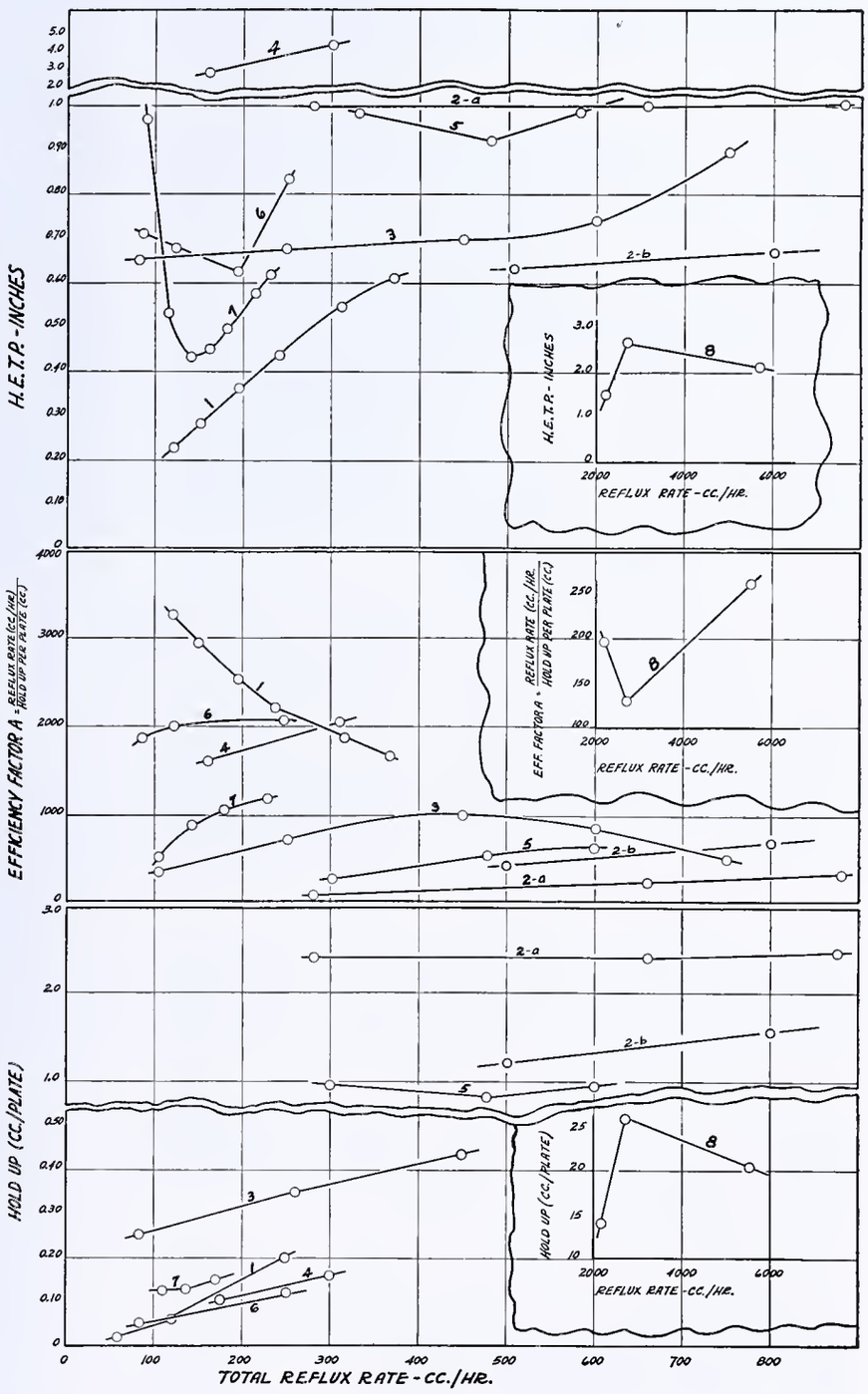
H.E.T.P.-INCHES
EFFICIENCY FACTOR A = REFLUX RATE (CC./HR.) / HOLD UP PER PLATE (CC.)
HOLD UP (CC./PLATE)
TOTAL REFLUX RATE-CC./HR.
REFLUX RATE-CC./HR.
EFF. FACTOR A = REFLUX RATE (CC./HR.) / HOLD UP PER PLATE (CC.)

FIGURE 7. COMPARISON OF HELI-GRID WITH OTHER HIGH-EFFICIENCY LABORATORY COLUMN PACKINGS

1. Heli-Grid, 11-mm. inside diameter, 35-cm. (14-inch) section
2-*a*. Fenske, single-turn metal helices, 1.98-cm. (0.79-inch) inside diameter, 277.5-cm. (111-inch) section (*4*, *17*)
2-*b*. Fenske single-turn helices, 1.55-cm. (0.62-inch) inside diameter, of No. 34 stainless steel wire, 25.4-mm. inside diameter packing, 100-mm. length of packed section (*18*
3. Purdue, spiral screen, 1.875-cm. (0.75-inch) inside diameter, 42.5-cm. (17-inch) section (*6*)
4. Spinning band, 6.7-mm. inside diameter, 540-cm. (216-inch) section (*1*)
5. Bruun 100-plate bubble cap, 195-cm. (78-inch) section (*3*)
6. Burk-Selker, concentric tubes, 14-mm. inside diameter, 150-cm. (60-inch) section (*14*)
7. Stedman, conical wire cloth, 9.5-mm. inside diameter, 60-cm. (24-inch) section (*16*)
8. Urey, rotating cone, 10.5-meter (35-foot) column (*5*)

developing the packings and it was gratifying to find that considerable improvement resulted from this procedure. Once wetted by preflooding, a packing will operate at its best efficiency throughout a distillation, until dried out by stopping distillation or otherwise.

Heli-Grid packing sizes have been worked up from 4.5-mm. (used for low-temperature fractionation) to 25-mm. diameter. Unlike certain other types of high-efficiency packings, the H. E. T. P. and factor A curves for the Heli-Grid type of packing do not deteriorate with diameter for the sizes thus far developed. This is as expected, as the design of diameter packings is varied to maintain vapor passageway length and clearances, etc., as constant as possible for all the packing sizes.

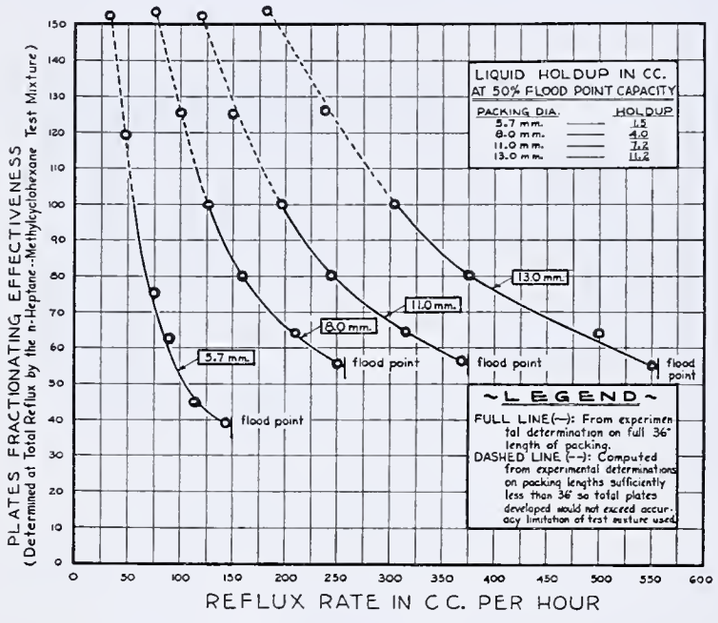
PLATES FRACTIONATING EFFECTIVENESS
(Determined at Total Reflux by the n-Heptane--Methylcyclohexane Test Mixture)
REFLUX RATE IN C C. PER HOUR
LIQUID HOLDUP IN CC.
AT 50% FLOOD POINT CAPACITY
PACKING DIA. HOLDUP
5.7 mm. 1.5
8.0 mm. 4.0
11.0 mm. 7.2
13.0 mm. 11.2
5.7 mm.
8.0 mm.
11.0 mm.
13.0 mm.
flood point
~LEGEND~
FULL LINE (—): From experimental determination on full 36" length of packing.
DASHED LINE (– –): Computed from experimental determinations on packing lengths sufficiently less than 36" so total plates developed would not exceed accuracy limitation of test mixture used.

FIGURE 8. TYPICAL PERFORMANCE CURVES FOR HELI-GRID PACKINGS OF VARIOUS DIAMETERS

The above performance results for the Heli-Grid packing are not to be considered at all final or maximum. It is expected that refinements in design and construction will result in enhancing performance and especially in lifting the low end of the performance curves at high reflux rates.

Application of Column and Packing

TO FRACTIONATION ABOVE ROOM TEMPERATURE. With the new type of column and packing, fractionation of normally liquid mixtures above room temperature differs from low-temperature fractionation in only two ways: (1) the sample may and usually should be much larger in the case of high-temperature fractionation, and (2) the upper portion of the distilling tube and the reflux cooling head are suitably designed for handling components condensing above room temperature and collected in the liquid state.

Figure 3 illustrates primarily adaptations of the Heli-Grid packed Super-Cal column to high-temperature fractionation. In the vacuum-jacketed distilling flask series, a heater well and a cartridge type of heater are used, preferably with a suitable heat-transfer liquid, such as oil, filling the heater well. If this seems inadequate to one accustomed to piling on heat on the usual non-jacketed distilling flask, it should be remembered that only a fraction as much heat need be supplied through the walls of the vacuum-jacketed flask.

Figure 8 gives the performance characteristics of Heli-Grid packed Super-Cal columns, as determined by the *n*-heptane and methylcyclohexane test mixture. The packings differ mainly in distilling capacity and holdup. Best results cannot be obtained from any packing without proper thermal surroundings and close control over all distillation factors.

Figure 9 shows a complete fractionation apparatus with a Super-Cal type of column and vacuum-jacketed distilling flask, with an exact rheostat and wattmeter system for controlling and measuring heat input to flask, and with a sensitive vacuum control for use in vacuum distillation.

In this apparatus, the Heli-Grid packed Super-Cal column has been tested on a wide variety of distillable mixtures, such as high-boiling petroleum fractions, heat-sensitive essential oils, fatty acids, etc. The Heli-Grid packing loses comparatively little of its distilling capacity even down to 20-mm. absolute pressure, presumably because its bottleneck is liquid rather than vapor volume rate. Owing to its wire structure, it is possible to drain absolutely all liquid from packing towards the end of a distillation, by chilling the flask.

One interesting phenomenon observed in this work deserves at least passing mention, although full discussion must be reserved until completion of quantitative tests now in progress. It has long been tacitly assumed that the best way to operate any column is with as nearly continuous a product-withdrawal rate as possible. The computation of column plates and obtainable separations as by the Smoker equation (*15*) uses the reflux ratio obtained by dividing reflux rate by average product rate.

However, in operating the high-efficiency column and packing here described, on close-boiling binary test mixtures at high reflux ratios, very much better separations have been obtained by holding the column at total reflux for periods of 30 minutes or more, and then withdrawing a quota of product almost instantaneously, again holding the column at total reflux, and so on, than by withdrawing the product continuously at the same average rate as in the intermittent operation. In fact, the separations obtained are better than called for by either Rose's computations of batch fractional distillation curves (*13*) or Smoker's formula (*15*). The same phenomenon has long been observed in experimental work on

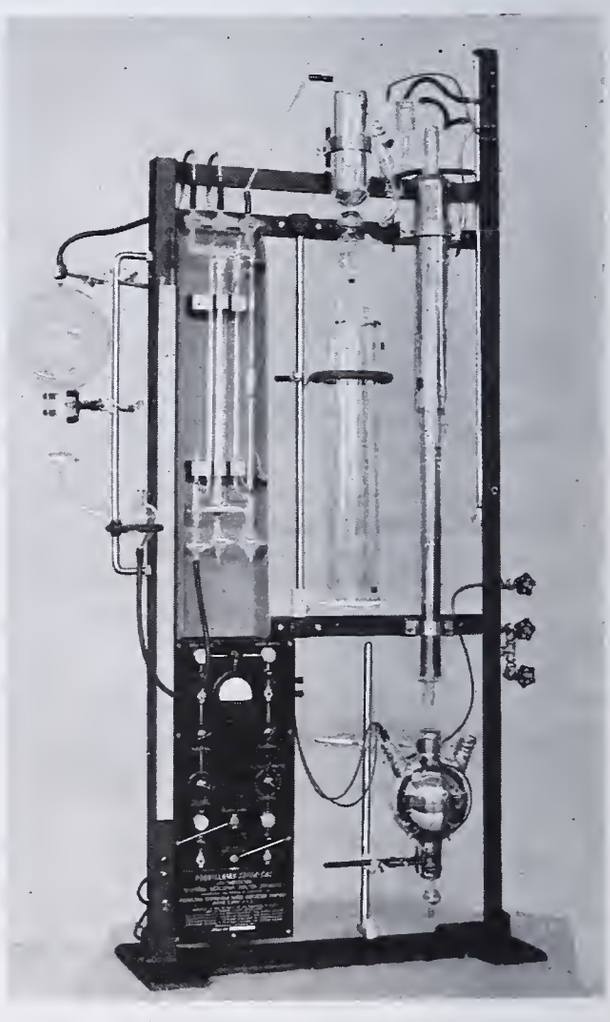

FIGURE 9. COMPLETE HIGH-TEMPERATURE FRACTIONATING APPARATUS WITH HELI-GRID PACKED SUPER-CAL COLUMN AND VACUUM CONTROL

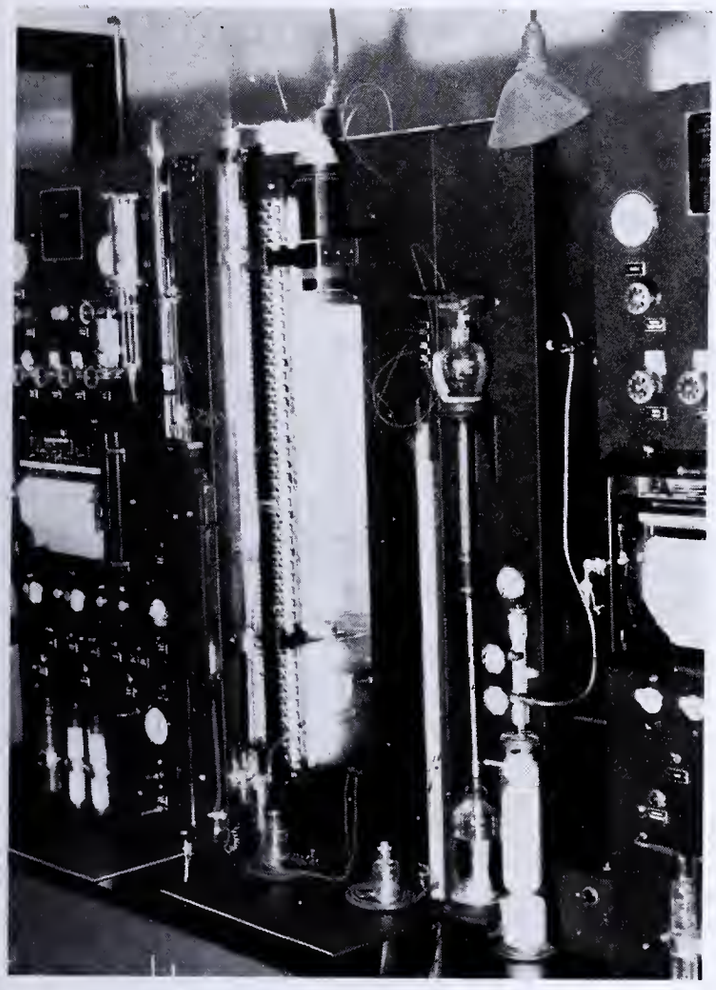

FIGURE 10. HYD-ROBOT AUTOMATIC RECORDING LOW-TEMPERATURE FRACTIONATING APPARATUS WITH HELI-GRID PACKED SUPER-COOL COLUMNS

centrifugal fractionators, and is now understood to be a factor in low-temperature fractionation. Like the nonadiabatic column performance phenomena mentioned above, this unexpected advantage of intermittent product withdrawal, so outstanding as to merit the nickname "the free-wheeling effect" in the author's laboratories, still is without theoretical explanation.

In application to design of the Super-Cal column, the "free-wheeling effect" makes desirable as close and accurate control of reflux cooling as possible, with lowest possible holdup in the condenser. This is contrary to the tendency displayed in the literature to use complicated reflux distributor heads of high vapor and liquid holdup.

APPLICATION TO HIGH-EFFICIENCY LOW-TEMPERATURE FRACTIONATION. The most immediate and effective use of Heli-Grid packings within the Super-Cool type of low-temperature fractionating column (*12*) has been for the complete analysis of all the components of the cracked C_4 fraction of refinery gases, by combined high-efficiency low-temperature fractionation and supplementary chemical tests. The separation of isobutane from isobutene, and the separation of butene-1 from *n*-butane, are exceedingly difficult, and require both in theory and practice more than 50 theoretical plates and a reflux ratio higher than about 100 to 1 to obtain satisfactory separation. The wire-coil packing and its simple modifications, while satisfactory for the easier separations of hydrocarbons occurring in natural gas and gasoline (*8*), do not have sufficient fractionating power to separate components boiling as close together as 6° C., even at total reflux. On the other hand large-diameter high-holdup packings are manifestly not usable for the precise analysis of samples of condensate as small as 2 cc. of liquid.

As a matter of fact the need for a high-efficiency low-temperature packing to make complete analysis of the cracked C_4 fraction possible had become both urgent and serious, inasmuch as daily analyses of this type are a practical necessity for the efficient operation of alkylation plants for the production of high-octane aviation gasoline stock, and in catalytic cracking and other modern refining processes. The development of the Heli-Grid packing was undertaken with this exigency in mind. At this writing the Heli-Grid packing is used for complete cracked C_4 analysis in many alkylating and catalytic cracking plants in this country.

Figure 10 shows an automatic recording low-temperature fractionating apparatus, using Heli-Grid packed Super-Cool columns for the routine daily analysis of alkylate gases for efficient plant control. Apparatus of this type and their operation and performance are described elsewhere (*11*, *12*).

When applied to fractionation of natural gas and gasoline, the Heli-Grid packing makes possible the sharp separation of isobutane from *n*-butane at distillate rates of the order of 40 to 60 cc. of gaseous products per minute, as against approximately 10 to 15 cc. per minute maximum for the single wire-coil packing. A similar improvement in distillate rates has been noted for the separation of ethylene from ethane and of isopentane from *n*-pentane.

Either the Super-Cool or Super-Cal column with Heli-Grid packing thus serves as a precision microfractionating column testing from 50 to 100 plates, depending on operation, filling a need for improved high-efficiency low-temperature fractionating columns to meet the new analytical requirements of the petroleum and other industries.

Acknowledgment

Flexible glass bellows expansion joints of suitable deflection and mechanical characteristics for use in vacuum jackets were developed by Morley R. Corbett of the author's laboratory, originally in collaboration with D. J. Pompeo.

The cooperation of Alfred Viergutz of the author's laboratory on this project has been especially helpful in the development of Heli-Grid packing.

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PRESENTED before the Division of Petroleum Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Mo. This paper is the fifth of a series of which four have been published (*8*, *9*, *10*, *11*) and another is in press.

Multiple Electrode System for Potentiometric Titration Studies

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FOR the practical application of potentiometric titration methods to industrial analyses, it is highly desirable to select with care the electrodes to be used in following the course of the reaction.

A relatively large change in potential with a small change in concentration should occur in the vicinity of the equivalence point; up to the vicinity of the equivalence point the potential difference between the electrodes should remain relatively constant; the indicator electrode should reach equilibrium rapidly, so that the course of the reaction may be followed closely; and the electrodes used should not be subject to poisoning nor be attacked by the solution during the titration.

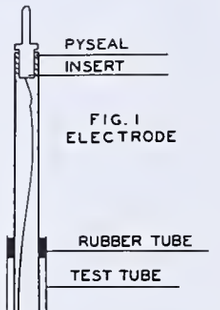

FIG. 1
ELECTRODE

In investigating practical applications of potentiometric procedures in this laboratory it has been found expedient to make use of a flexible multielectrode system with which it is possible to determine the optimum system to be used for any specific determination under the exact conditions obtaining.

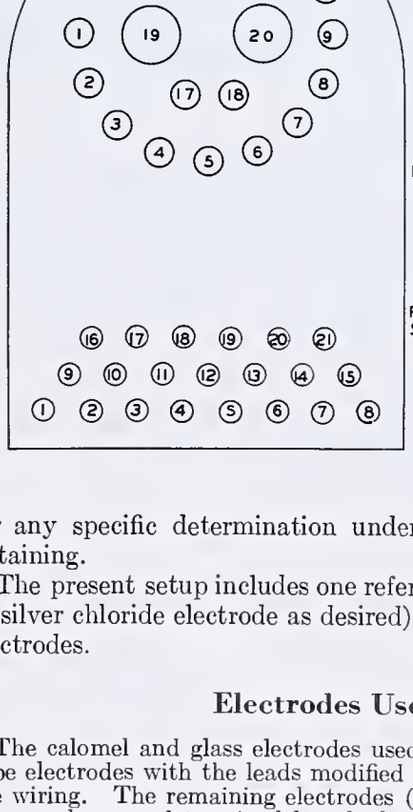

FIG. 2
ELECTRODE SUPPORT TEMPLATE

The present setup includes one reference electrode (calomel or silver chloride electrode as desired) and eighteen indicator electrodes.

Electrodes Used

The calomel and glass electrodes used are standard Beckman type electrodes with the leads modified to fit in with the rest of the wiring. The remaining electrodes (Figure 1) were prepared in several ways, determined largely by the physical limitations of the materials.

When possible (antimony, bismuth) the pure or purified metal was cast into a cylindrical rod (about 1 cm. in diameter, 5 cm. long) with a piece of copper wire (for lead) imbedded in one end. A piece of Pyrex glass tubing usually sufficed for the mold. The rod was then machined to the shape indicated in Figure 1 and sealed into a piece of glass tubing (7 mm. in outside diameter 12 cm. long, ends ground) with Sauereisen cement No. 31. The copper wire lead was soldered to a brass insert, sealed to the opposite end of the tube with Pyseal cement, a 15-cm. length of push-back wire was in turn soldered to the brass insert, and a pin tip was connected. For metals which could not be cast (platinum, tantalum) a short length of pure wire was welded to the copper lead and then sealed into the glass tube as above. If possible, the wire electrode was coiled to expose greater surface. The machined electrodes were highly polished. A 1-cm. length of 0.47-cm. (0.19-inch) bore Scimatco rubber tubing was cut and slipped onto the glass tube as shown, so that a standard 11 × 75 mm. test tube could be slipped over the end of the electrode tip and kept in place by the rubber tubing. With the test tube in place an electrode may be shielded from contact with corrosive solutions. Solutions for preservation or cleaning of any electrode may be placed in the test tube and the electrode stored in favorable media when not in use without removing it from the electrode support.

Electrode Support

The electrodes were mounted in a Bakelite board cut and drilled as indicated in Figure 2. They were arranged on the periphery of a circle 7 cm. in diameter, so that with all electrodes and their shielding test tubes in place a 600-ml. beaker in which titrations

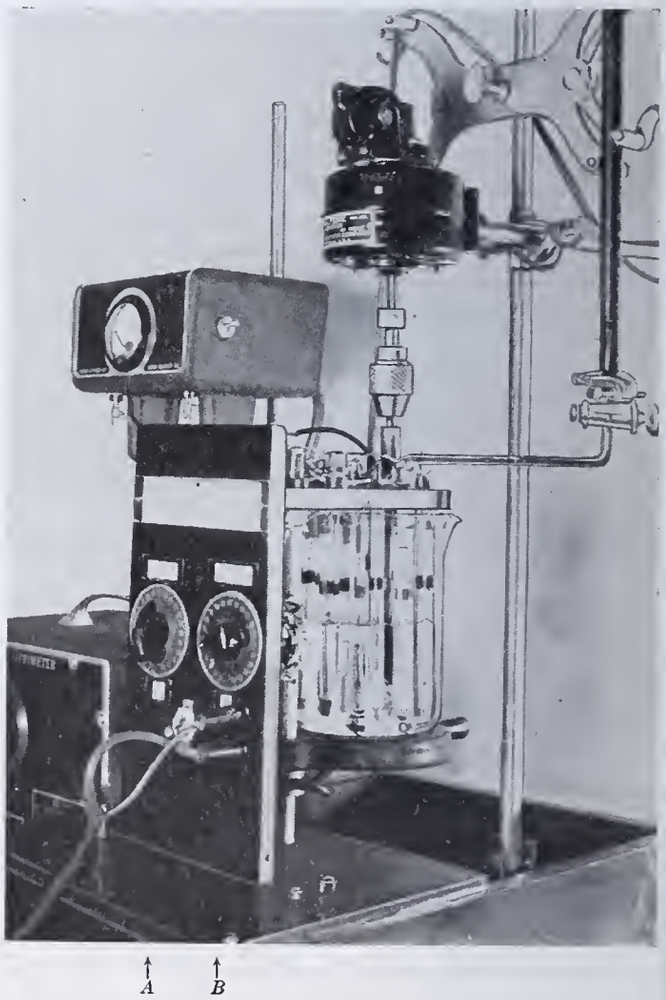

FIGURE 3. APPARATUS

may be conducted could be raised to surround them. Provision is also made in the Bakelite board for an all-glass stirrer and modified buret tip (Figure 3). In mounting the electrodes in the Bakelite board a 0.62-cm. (0.25-inch) hole was drilled through, then beveled to 0.94 cm. (0.375 inch) on the upper side. The electrode was inserted so that the top of the brass insert projected through the board and sealed in place by filling the beveled region around the glass with Pyseal. The electrodes are thus held firmly but may easily be removed by warming the cement with a hot file or small soldering iron.

Electrode Assembly

Two Centralab 23 position selector switches were mounted on the front panel and the common terminal of each was connected to individual insulated jacks (one red, one black, Figure 3) through a double-pole double-throw toggle switch. A series of jacks (taking phone tips) was then mounted on the electrode support (20 in this particular instance) and each jack was connected to the same position on both switches. Thus when the antimony electrode was installed the tip from the antimony lead was inserted in jack No. 2 and the latter in turn connected to position 2 on switches *A* and *B* (Figure 3). The calomel electrode was plugged to jack No. 5 and the latter connected to position 5 on switches *A* and *B*. The electrode leads from the titrimeter used were plugged into the red and black jacks on the front panel.

Thus, with the electrodes immersed in the solution to be studied, by setting switch *A* to position 2 and *B* to 5, with the toggle switch in the up position, the antimony electrode is connected to the red jack and the calomel to the black jack (and in turn to the titrimeter). Flipping the toggle to the down position immediately reverses the polarity, connecting the antimony electrode to the black and the calomel to the red plug. Reversal of polarity could be obtained, of course, by leaving the toggle in the up position and turning switch *A* to 5 and *B* to 2. With this flexible arrangement any pair of electrodes could be used and the polarity with respect to the titrimeter reversed at will. By including a standard reference electrode (calomel half-cell) comparative studies are simplified.

A glass electrode was included for ease in adjustment of solution pH. For example, the effect of acid concentration on a reaction may be investigated by connecting the glass and calomel electrodes to the titrimeter, adding acid from a buret until the desired pH is attained, connecting the desired indicator electrode (or electrodes) to the titrimeter by readjustment of switches *A* and *B*, and titrating with the standard reagent solution. At any point during the titration the initial electrodes may be reconnected to the titrimeter by adjustment of *A* and *B* and the pH checked rapidly.

A list of the electrodes used, with their position on selectors *A* and *B*, is mounted on the front panel for convenience. The nature of the electrodes to be used for any one study depends upon the reaction to be investigated. With this setup the best electrode pair for titration of any solution, following the five considerations outlined above, may be readily and quickly determined.

A Shaping Lathe for Graphite Electrodes Used in Spectrochemical Analysis

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THE need for a rapid and convenient means of shaping graphite electrodes for use in spectrochemical analysis of plant ash led to the design and construction of a small portable shaping lathe. This consists of two essential units, a lathe head and an electrode feeding assembly, securely held in proper relative position on a base plate.

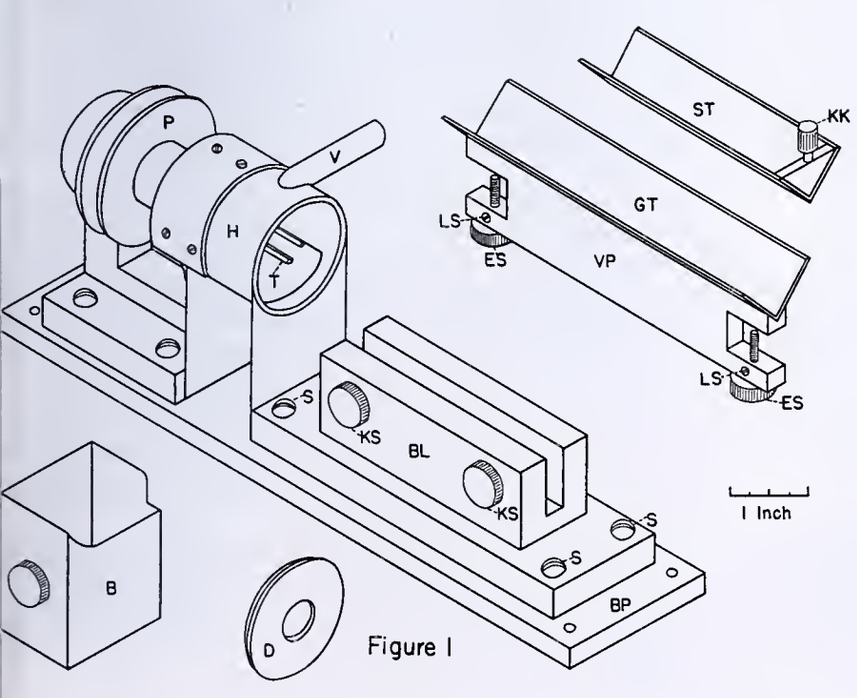

Figure 1

Lathe Head

A motor-driven shaping bit mounted horizontally, and an enclosure for guarding the cutting end of the bit and collecting the graphite trimmings, are the main elements of the lathe head (Figures 1 and 2). The outer section of the shaping bit (*T*, Figure 2) forms the shaft of the rotating part and turns in two snugly fitting dustproof ball bearings (*BB*, Figure 2). A pulley wheel locked to this shaft between and in contact with the two bearings prevents thrust play in the shaping bit. The support for bearings and shaft is fastened securely to the main base plate.

A shallow cylindrical housing encloses the cutting end of the bit. A section of the underside cut away allows heavier graphite trimmings to drop into a removable bin (*B*, Figure 1). Lighter graphite dust is removed by suction through a tube fastened in the cylinder top. If the type of shaping or trimming operation does not produce large graphite chips or shells, the bin and bin enclosure can be omitted and the suction port placed on the underside of the cylindrical housing. The vacuum system will keep graphite dust from collecting.

A removable flanged disk (*D*, Figure 1), with a central opening, fits into the open end of the cylindrical housing. The central opening, while large enough to allow the entrance of the largest diameter electrode used, permits neither the operator's fingers nor the electrode carrier to come in contact with the rotating bit.

Electrode Feeding Assembly

The slide trough (*ST*, Figure 1) for carrying the electrode, the guide trough (*GT*, Figure 1) fastened to a vertical plate, and a slotted metal block (*BL*, Figure 1) make up the electrode feeding

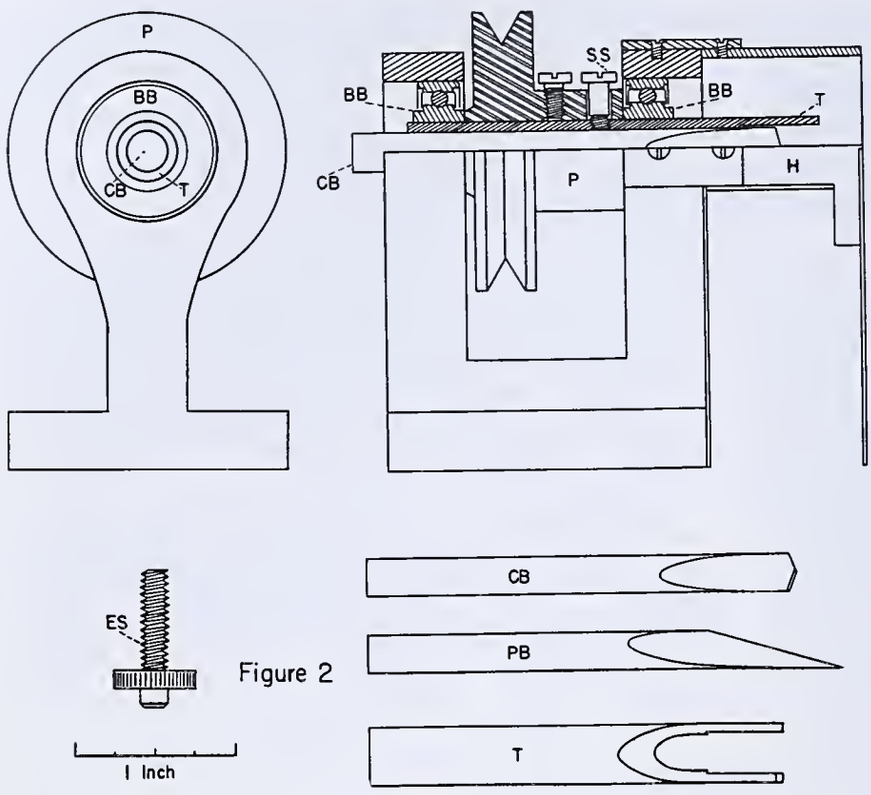

Figure 2

assembly. The sliding trough has the end away from the lathe head closed by a metal block. By means of knurled screws (*KS*, Figure 1) the vertical plate of the guide trough is clamped against an inner wall of the slotted block in such a manner that the line formed by the angle of the trough lies parallel and directly below an extension of the shaping bit axis. This forms a rigid guide for the slide trough. The contacting surfaces of the two troughs are machined true to prevent rocking or side play.

The flat plate holding the slotted block is fastened to the main base plate at each corner by steel screws. The screw holes in the upper plate are slotted at right angles to the shaping bit axis to allow sufficient lateral alignment of the guide trough. This lateral adjustment should be required only when the lathe is first assembled.

The vertical adjustment of the guide trough, necessary whenever the diameter of the electrode material is changed, is made by movement of the steel elevator screws (*ES*, Figures 1 and 2) located in each end of the vertical plate. Accidental alterations in screw settings are prevented by brass setscrews (*LS*, Figure 1). The length of the elevator screws determines the maximum and minimum electrode diameters which can be centered on the shaping bit axis.

The completed lathe assembly, a 0.05-horsepower motor which drives the shaping bit at about 1700 r. p. m., and a control switch are mounted on a wooden base.

A water aspirator supplies sufficient vacuum to collect the fine graphite dust. To prevent graphite from clogging the vacuum line or aspirator, a trap located on the wooden base near the lathe head is provided.

Shaping Tools

A variety of sizes and designs of shaping tools can be interchanged readily in the lathe described. Size and design are limited only by inside diameters of the pulley wheel and bearings, and dimensions of the cylindrical housing enclosing the bit end. Shaping bits and drills with diameters smaller than the maximum fixed by pulley and bearings can be accommodated by using sleeves or holders as adapters.

The shaping bit used in this laboratory was made according to the design and dimensions described by Myers and Brunstetter (*2*) for forming shallow, thin-walled craters in 0.31-inch graphite electrodes. In using this shaping bit in the shaping lathe, the blunt end of the inner drill (*CB*, Figure 2) is made to extend beyond the blunt end of the outer trimmer (*T*, Figure 2). By changing the length of the shaft which protrudes, craters of varying depths can be made.

Certain fixed depths of craters are obtained by using gages to set the position of the shaft. The gages are short pieces of 0.375-inch brass rod, each of which has a hole of appropriate depth drilled in one end. The holes permit passing the gages over the blunt end of the inner drill, but not over the end of the outer trimmer. The inner drill, released by turning setscrew *SS* (Figure 2) which extends through the pulley collar and outer trimmer, is seated into the outer trimmer as far as the specific gage permits.

To point the upper electrodes, a special bit (*PB*, Figure 2) is used which is interchanged with the inner drill of the shaping tool used for cupping electrodes. It is set into correct position by the use of a gage similar to ones just described.

The Chemistry Department of the University of Illinois has made further variations in shaping tools used with a copy of this lathe. For spectrochemical work done there, a facing tool and two small-diameter drills are required. Types of electrodes produced are described in an account of the spectrochemical technique used by Keirs and Englis (*1*).

Operation

For the shaping operation, the electrode is placed in the carrier with one end extending about 0.75 inch beyond the end of the sliding trough. The other electrode end lies against the closed end of the trough. While the electrode is being held down firmly in the trough by manual pressure, the carrier is moved slowly into the shaping bit by means of the knurled knob. The slide is drawn directly back to remove the shaped electrode from the cylindrical housing. Electrodes of shorter length can be accommodated by using spacers placed between the electrode and the closed end of the trough. Electrode material cut into appropriate lengths is suitable for this purpose.

Frequent rim breakage of the cupped electrodes during the shaping operation can be traced to several possible causes, such as the guide trough not in proper alignment, shaping bits which are not machined true, dull cutting edges, and low bit speeds. According to Myers and Brunstetter, bit speeds below 1300 r. p. m. cause excessive breakage.

The principal features of the lathe are the speed, accuracy, and convenience with which electrodes may be shaped. Eight or twelve cupped electrodes may be prepared per minute. Speed has been increased by omitting the operations of clamping and unclamping electrodes in a chuck or other similar device.

Accidental contamination of cutting surfaces of the shaping tools by dust or indiscriminate handling is prevented by the cylindrical housing. It is possible, therefore, to keep the shaping lathe on the bench where samples for spectrochemical analysis are prepared. Since the tool is not adapted to other sundry uses, contamination from miscellaneous usage is avoided.

With the lathe immediately accessible, and because its manipulation is not difficult, there is no necessity for preparing a large number of electrodes at one time and storing them for future use. The danger of rim breakage or contamination which often accompanies storage is eliminated by shaping only enough electrodes for immediate use.

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The laboratory is a cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

An Apparatus for High-Speed Stirring

Effect of Flask Design and Some Other Factors on Stirring

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STIRRING is one of the most common practices in the laboratory and is indispensable for many reactions. Its benefits increase (*1*, *2*, *8*) as the velocity increases: Some reactions indeed occur to an appreciable extent only when the velocity becomes very great (*4*, *5*). In spite of the manifold uses to which stirring has long been put and the obvious advantages of high speed and efficiency, there has been only one apparatus (*4*) designed for operations at 10,000 r. p. m., of such construction as to be unsuitable for everyday laboratory use, and little, if any, serious study has been devoted to factors such as flask shape, stirrer design, etc., requisite for maximum effectiveness. Possibly the complexity of a subject in which an unusually large number of variables exists has deterred many would-be investigators, but the likelihood of greatly improving the yields for many known reactions and of discovering new reactions warrants a real effort to acquire knowledge of ideal conditions in an old art.

This paper gives directions for constructing a laboratory stirring apparatus which can operate at speeds near 11,000 r. p. m., yet is so simple that it can be mounted on an ordinary ring stand. Results of some studies on the type of flask suitable with stirrers of the propeller type at relatively high speeds are recorded. A simple method for comparing relative efficiencies of flasks, stirrers, and other factors has been developed. It is shown conclusively that a change in flask design has a direct bearing on efficiency of stirring and that the best flask is one from which swirling, obstructions, and pockets are largely eliminated.

High-Speed Stirring Apparatus

The apparatus consists of a motor mounted with its shaft in a vertical position, a stirrer of the propeller type supported in ball-bearing assemblies, and a flask. In all the experiments reported in this paper, a 0.04-horsepower series-wound ball-bearing motor (G. E.) with rated speed at 10,000 r. p. m. was used. It is bolted to a 0.94-cm. (0.375-inch) steel block into the center of which a 1.25-cm. (0.5-inch) steel rod is screwed, so that the motor is supported from an ordinary clamp fastener. This motor is satisfactory for many ordinary uses but lacks power to stir some mixtures at the highest speeds.

The equipment has since been supplemented by addition of a 0.25-horsepower series-wound ball-bearing motor also with a rated speed of 10,000 r. p. m., and mounted similarly on a 1.25-cm. (0.5-inch) steel block but supported by two instead of one rod and clamp device (Figure 1). The total weight of the large motor, block, and rods is about 3.7 kg. (10 pounds). The last unit is recommended for general use, since it is sufficiently powerful to stir at high speeds nearly all mixtures met in laboratory practice.

The propeller shaft is a 0.6-cm. (0.25-inch) stainless (18–8 alloy) steel rod 37.5 cm. (15 inches) long, supported by two short sections of pressure tubing (*3*) in two ball-bearing assemblies (about 2.2 cm., 0.875 inch, in outside diameter) which are placed about 15 cm. (6 inches) apart. The end-to-end contact between propeller shaft and motor shaft is closed by a short length of pressure tubing which is slipped over each end and held by a couple of turns of wire. Perfect alignment of motor and propeller shaft is not required in this arrangement.

A holder for the ball-bearing assemblies is made from two 17.5-cm. (7-inch) lengths of angle iron, the larger one 3.75 cm. (1.5 inches), and the smaller one 2.5 cm. (1 inch), held together tightly (Figure 1) by four machine bolts passed through both pieces. A little milling was necessary on the smaller angle iron in order to make it fit tightly against the ball-bearing assembly. Two sections of 1.25-cm. (0.5-inch) iron rod are tapped into the larger angle iron to support the holder in two clamp fasteners.

The propeller is made from a 5-cm. (2-inch) square piece of stainless sheet steel, No. 18 gage. A 0.3-cm. (0.125-inch) hole is first drilled in the center. The 0.6-cm. (0.25-inch) shaft is then turned down to 0.3-cm. (0.125-inch) diameter for a distance of 0.3 cm. (0.125 inch) at one end. The rod is next fitted into the hole and the two are riveted together by a few good blows with a hammer while the rod is held upright in a vise. This attachment is firm enough to permit mounting the shaft in a lathe and cutting the head to a diameter (slightly less than 2.5 cm., 1 inch) which will just pass through the neck of the flask. The disk is then slotted radially as many times as desired with a hacksaw and the segments are twisted at an angle of 45° to the shaft.

A completed assembly is shown in Figure 1. The apparatus runs smoothly and without appreciable vibration. The base of the ring stand can be anchored to the desk with screws or C-clamps, but this precaution is usually unnecessary. The noise is not unduly great, considering the high speeds involved.

The apparatus is operated with a variable transformer of 5-ampere capacity in series with the motor. The revolution rate is measured by a stroboscope (Strobotac) whose light is fixed on a marked metal disk attached to the shaft.

The small motor becomes very hot, particularly on long runs, but a blast of air directed into an opening on the underside cools it effectively. A cotton filter on the air line is usually required to remove grease.

At speeds of 10,000 r. p. m. some liquid may be forced out through the glass bearing unless it is fitted very well. In later work the authors were greatly indebted to Mr. Davidson for his suggestion of a very effective but simple rubber seal. A section of rubber tubing, 0.6 cm. (0.25 inch) in outside diameter by 0.94 cm. (0.375 inch) long, is pushed over the lower end of the glass bearing until the overhanging end of the rubber rests lightly on the propeller shaft. In this way the escaping liquid presses against the rubber and seals it more tightly. Another solution of this problem is to sink the neck far enough in the top of the flask to

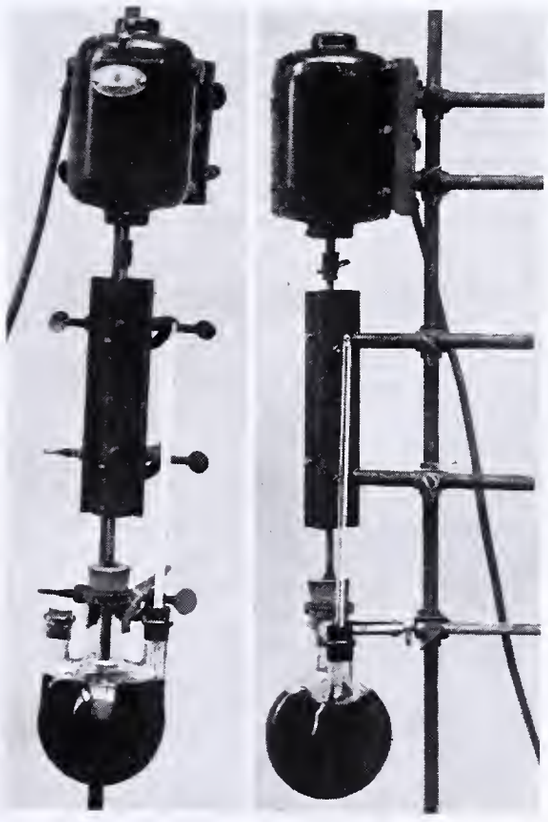

FIGURE 1. HIGH-SPEED STIRRING ASSEMBLY

FIGURE 2. AIR AND WATER MIXTURE STIRRED IN ORDINARY FLASK

make a ring seal that directs the liquid downward. This method is very satisfactory, although the effectiveness of the flask as a vehicle for stirring appears to be reduced considerably.

Metal instead of glass propellers are used because the latter are considered dangerous at high speeds. There have been several instances in this laboratory, however, when glass has been used at 5000 r. p. m. and even higher. The glass stirrer was usually constructed from 0.6-cm. (0.25-inch) or larger glass rod. In general, its use is not recommended unless necessary to avoid corrosion and then only with adequate safeguards to avoid injury if a section of the glass breaks and goes through the flask.

The apparatus as a whole is exceedingly convenient and easily constructed. It is a decided simplification over the one previously described (*4*) which included a substantial iron pipe framework, numerous pulleys and belts, and a special location.

Flask Design and Other Factors

The performance of this apparatus, using a stirrer of the propeller type, was observed in several series of experiments in which the type of flask, number of propeller segments, position of propeller, and a few other variables were studied. Major interest centered in the design of the flask. A special flask having deep vertical creases (*6*, *7*) has been responsible for a considerable increase in yield in many reactions and, in one case (*7*), for eliminating altogether a certain product which was obtained in an ordinary flask.

The comparative efficiency of this special flask over the common one in mixing air and water can be observed visually in Figures 2 and 3, taken with an Edgerton high-speed flash lamp, with exposure of $^1/_{30,000}$ second. In both pictures, the propeller was moving at 11,000 r. p. m. All conditions were identical save for the type of flask used. In Figure 2 the characteristic swirling and vortex are plainly visible. Beneath the propeller is a relatively quiet place in which little mixing occurs. The two bright spots on either side of the flask are due to reflections from the photographic lamp. The little ring on the propeller shaft above the liquid surface is composed of drops of liquid which have fallen on or climbed up the shaft. Figure 3 shows the effect in the creased flask. Uniform mixing is secured. The positions of the creases are shown by the reflections as bright lines of the edges. The circular spot a little below the neck is the roughened place for pencil marking present on all commercial flasks.

In addition to this visual evidence, it was found that the oxidation of toluene by permanganate at 10,750 r. p. m. for 30 minutes in the ordinary flask was so slight as to produce no temperature rise over the 1.6° C. due to mechanical agitation. With a creased flask other factors being constant, a total temperature rise of 12.3° was noted, of which 7° was the result of chemical heat.

The unusual successes which have attended use of this creased flask warranted study of the effect of additional variations from this type. The four varieties considered in this study are labeled as:

O An ordinary 3-necked flask with four vertical creases
S Same as *O* but with powdered glass sintered to the bottom in order to act as an abrasive
I Same as *O* but with the bottom pushed inward about 2.5 cm. (1 inch) in the form of an inverted funnel which is about 4.4 cm. (1.75 inches) in diameter at the bottom
P Same as *O* but with the bottom pushed outward in the conventional pear shape

The *O*, *I*, and *P* flasks are pictured in Figure 4. All were made from commercial 500-ml. 3-necked Pyrex flasks. Four vertical creases were made in each to a depth of about 2.5 cm. (1 inch) at the deepest point. Save for the four distinctions made above, construction of each was as nearly identical as could be accomplished by a competent glass blower. A large Thiele tube was also used as a flask in a few experiments.

Measurement of Efficiency

Efficiency was judged on the basis of the temperature rise, starting from room temperature, with the vessel shielded by

FIGURE 3. AIR AND WATER MIXTURE STIRRED IN CREASED FLASK

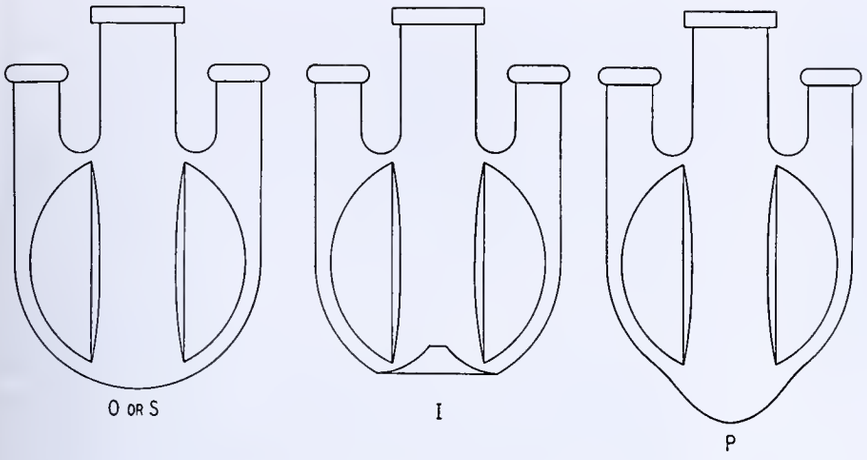

FIGURE 4. TYPES OF FLASKS USED IN TESTS

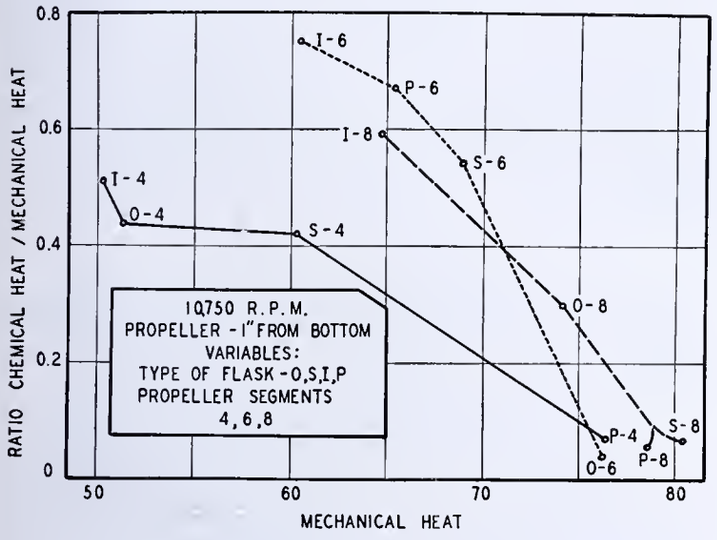

FIGURE 5. EFFECT OF VARYING FLASKS AND STIRRERS AT 10,750 R. P. M. AS TESTED BY EVOLUTION OF HEAT IN OXIDATION OF TOLUENE

an insulated can. The reaction was the permanganate oxidation of toluene (at 10,750 r. p. m.) or xylene (at 6000 r. p. m.) over a 30-minute period. Part of the total temperature rise is due to the mechanical effect of stirring, so that an independent experiment, identical save for the absence of permanganate, was necessary. The respective areas on a time-temperature graph were then estimated. Numerical values for chemical and mechanical heats used in all graphs in this paper are merely these areas which are proportional to the true heat units. The total temperature increase was as much as 27° C. in the studies at 10,750 r. p. m.

This method has the great advantage of rapidity, so that a large number of observations can be made of many factors. Interpretation of data, however, is subject to the criticism that production of mechanical heat will automatically increase the total chemical heat according to the general expectation that a chemical reaction is doubled for every 10° temperature increment. Fortunately any such effect is negligible compared with the greater influence which the flask exerts on the efficiency of agitation.

Effect of Variables

Figure 5 pictures the ratio of chemical to mechanical heat plotted against mechanical heat for a study of the oxidation of 25 ml. of toluene by 2 grams of potassium permanganate in 300 ml. of water at 10,750 r. p. m.

The propeller was, in all cases, placed 2.5 cm. (1 inch) above the bottom of the flask. For the *I* flask, the bottom was taken to be the flat top of the conical indentation. The positions on the graph marked *I*-4, *S*-6, *P*-8, etc., refer to the test made on the *I* flask using the propeller with four segments, that in the *S* flask with the propeller having six segments, and that in the *P* flask with a propeller having eight sections. Lines have been drawn between points representing different flasks in which comparable conditions were present—for example, *O*-4, *S*-4, *I*-4, and *P*-4 are connected.

Points to the upper left represent high efficiency, a high chemical effect with a minimum of mechanical energy expended; while points to the lower right represent low efficiency, small chemical heat in spite of a relatively large mechanical effort. The results show clearly that in every series the *I* flask was superior. With one exception the pear-shape flask, *P*, was the most ineffective of the lot.

Lines could also have been drawn between points representing different propellers for otherwise comparable conditions—e. g., *I*-4, *I*-6, and *I*-8. In such a comparison the eight-bladed propeller gave poor results, although in every series but one it produced the largest mechanical heat. The performances of the four- and six-bladed stirrers were in general very good. If a line be drawn diagonally towards the northeast, so as to separate the six best from the six worst conditions, the *I* flask and the four-bladed propeller are represented three times each among the better group. These results are contrasted with the occurrence of the *P* flask (twice) and the 8-bladed propeller (three times) in the poorer half. The single occurrence of the eight-bladed propeller among the better group can be attributed to its being used with the best flask; likewise the single appearance of the four-bladed propeller with the poorer group can be explained on the ground that it was used with the poorest (*P*) flask. The results are in general striking, particularly in view of the later discovery (Figure 6) that the position of the stirrer at 2.5 cm. (1 inch) from the bottom was far from ideal.

Figure 7 shows a parallel study at 6000 r. p. m. of the oxidation of 25 ml. of xylene by 16 grams of potassium permanganate with 4 grams of sodium hydroxide in 300 ml. of water. The position of the propeller was again 2.5 cm. (1 inch) from the bottom. This figure exhibits the clear superiority of the

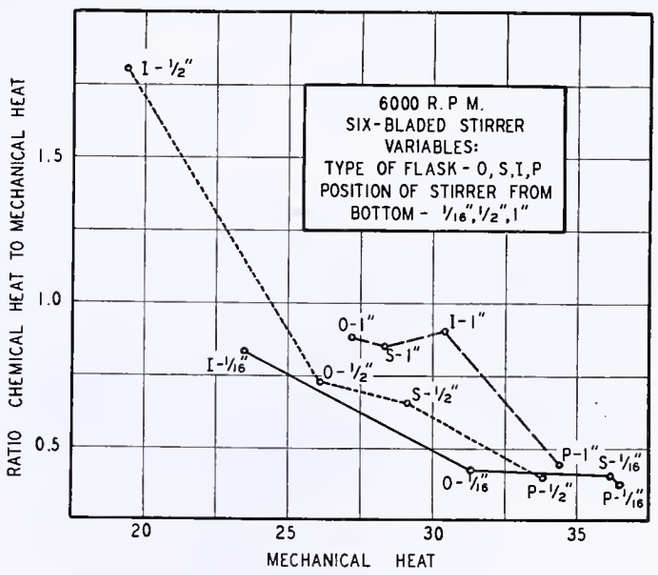

FIGURE 6. EFFECT OF VARYING POSITION OF STIRRER AS SHOWN BY EVOLUTION OF HEAT IN OXIDATION OF XYLENE

four-bladed propeller with every flask tried—(for example, compare *I*-4, *I*-6, and *I*-8 or *S*-4, *S*-6, and *S*-8) and the pronounced difference between the *I* and *P* flasks with this best (No. 4) stirrer. The series with the six-bladed propeller shows also a marked difference between these two flasks (*I*-6 and *P*-6) but the superiority of *I* over *O* and *S* is not so evident. It should be remembered, however, that the position of the stirrer in this set of experiments is not ideal for revealing any superior qualities present. Indeed, when the propeller is moved to a position 1.25 cm. (0.5 inch) from the bottom (Figure 6) other conditions being the same, the results with the six-bladed propeller confirm in every respect the decided superiority of the *I* flask over the three other types.

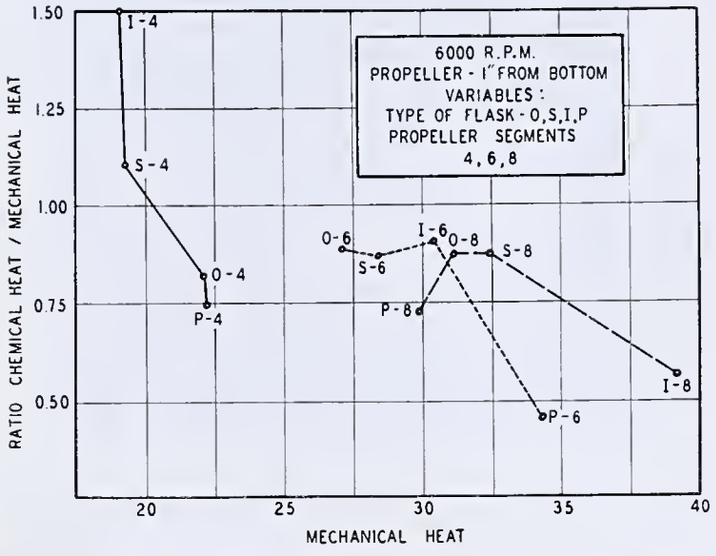

FIGURE 7. EFFECT OF VARYING FLASKS AND STIRRERS AT 6000 R. P. M. AS SHOWN BY EVOLUTION OF HEAT IN OXIDATION OF XYLENE

The erratic results (Figure 7) with the stirrer having eight segments are attributed both to its general ineffectiveness and unfavorable position. The mechanical heat generated by this stirrer, however, is 50 to 100 per cent larger than that realized by the four-bladed one.

Figure 6 depicts the effect of varying the position of the propeller with six segments at 6000 r. p. m. The same mixture was used as in the preceding test at this velocity. The results are decisive. The pear-shaped flask was, in all cases, the poorest of the lot. The position of the stirrer had little bearing on its effectiveness. The *I* flask, on the other hand, was the best in two out of three cases and not appreciably inferior in the third. The position of the stirrer had an immense effect on efficiency. When correctly placed 1.25 cm. (0.5 inch) above the bottom, a decided superiority resulted. In the light of this sensitiveness to position, the good showing of the *I* flask, in Figures 5 and 7, is all the more surprising.

The general improvement noted in the flask having the inverted bottom was confirmed by constructing two more of that general pattern but with slight modification. One had two deep intersecting creases across the bottom spaced between the creases in the side. The other had a number of Vigreux indentations in the lower half, the purpose of which was to promote more mixing by having the currents flow in and around the projections. Both innovations resulted, however, in some reduction in efficiency of the *I* flask, although still retaining a superiority over flasks *O*, *S*, and *P*. Even minor obstructions such as Vigreux points interfered with the proper functioning of the flask.

A further attempt to direct the flow of liquid inside the flask was made with the flask of the *I* type having its neck sunken so that the liquid would be forced down toward the propeller. For some reason not yet apparent, this innovation reduced the efficiency to a point where it was not much better than the pear-shaped flask. The change, however, did eliminate collection of liquid in the neck and any splashing through the stopper which sometimes occurred at the highest speeds.

A Thiele tube flask (from 3.4-cm., 1.375-inch, tubing) was also tested with the three propellers at 6000 r. p. m., using the same mixture and the same amounts employed for the creased flasks at that velocity. The volume of the tube was such as to be just filled by the mixture. Figure 8 shows that the mechanical input was considerably larger than before, the lowest value of 36 units in this series being near the highest value of 39 in Figure 7. So great was the power required to stir the mixture in this apparatus that it was impossible to attain a velocity higher than 7620 r. p. m. with the small motor used in all these tests. This series was designed to test the effect of introducing a screen, supported at a level 0.6-cm. (0.25 inch) below the propeller, which would break the current into small particles. A 16-mesh screen proved of little value, but one of 100-mesh showed a definite improvement. The propeller with four blades was again generally superior.

Interpretation of Results

As far as can be judged from this beginning study of a complicated problem, the following opinions may be ventured:

A. The role of the container in stirring appears to be that of furnishing a surface for rapid transfer of liquid away from and to a position above the propeller. In the ordinary round flask, the liquid swirls around the outside wall. The propeller throws the mixture outward and keeps it rotating but does not effectively mix the components. The creased flask directs the liquid flow upward, from whence it falls again upon the stirrer to be hurled once more in fast-moving currents from the propeller.

The additional superiority of a creased flask with an indented bottom is due to the further facilitation of movement of liquid within the flask. The effect is similar to that of streamlining. The pocket immediately below the propeller where liquid can collect and escape mixing is eliminated.

The poor results with the pear-shaped flask come from interference of the movement of the liquid. The propeller churns the liquid at the bottom, does a great deal of mechanical work in trying to force the liquid around an unnatural and difficult path, and is unable to perform its proper function of mixing because the flow of liquid is impeded.

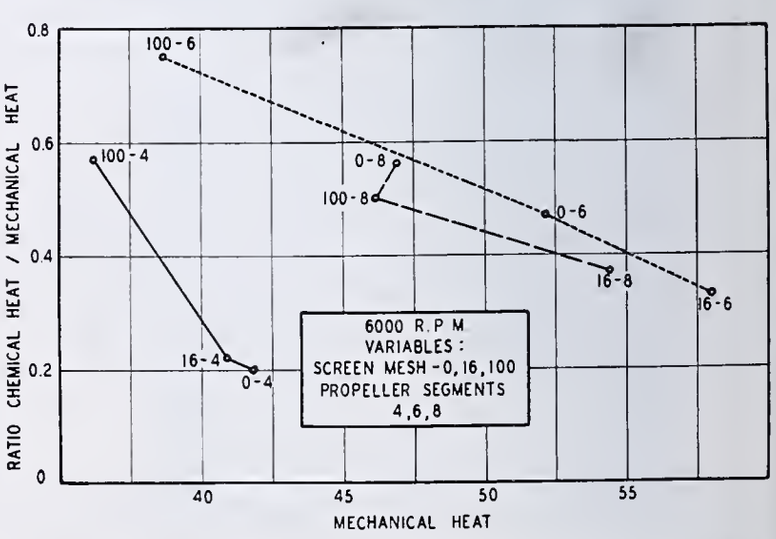

FIGURE 8. EFFECT OF VARYING STIRRERS AND SCREENS IN A THIELE TUBE AS MEASURED BY HEAT EVOLUTION IN OXIDATION OF XYLENE

B. The role of the propeller can be to generate fast-moving currents of liquid through the mixture. A propeller with a large number of blades operating at high speed might conceivably cut the liquid into many parts and accomplish a degree of mixing. Each segment, however, needs enough surface to serve as a good driver. The general ineffectiveness of the eight-bladed propeller at 6000 and 10,750 r. p. m. was pronounced. The mechanical heat generated by its use was in nearly every instance the greatest (sometimes by 50 to 100 per cent) of the three, yet it appeared to whirl around in a generally ineffective manner. The propeller with four blades gives a satisfactory performance. Even a good propeller, however, cannot translate a large amount of the energy it receives into useful work unless it is properly placed in a correctly designed container. Such principles might well be of practical importance in large-scale apparatus.

C. Impediments, whether points on the inner surface of the flask or walls to constrict the flow, result in a reduction in efficiency. A very fine screen which subdivides the liquid into small particles assists the reaction, but the energy required to operate a stirring apparatus of that type is very much greater and the ratio of chemical to mechanical heat is less than in a properly designed flask.

Acknowledgment

The authors are indebted to I. Amdur for very helpful suggestions in handling the data.

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Contribution from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology, No. 248.

Potentiometric Titration Stand Assembly

Louis Lykken and F. B. Rolfson, Shell Development Company, Emeryville, Calif.

THE use of electrometric titration methods has slowly become a common laboratory practice. Although potentiometric titrations have long been used by academic workers for special determinations, this type of analysis has found only limited application in many industrial laboratories because the apparatus has had to be assembled largely from miscellaneous parts at hand. Generally, this resulted in a cumbersome assembly which was inconvenient and unwieldy to use in routine determinations. Also, experience has shown that the use of loosely assembled equipment requires that a considerable part of the operator's time be spent in maintenance.

In designing the assembly described here, every effort was made to produce a titration stand which could be used for routine as well as special determinations. It was recognized that a satisfactory assembly must be as easy to use as the ordinary apparatus required for indicator titrations. The buret clamp, stirring motor, beaker cover, and electrode support have been semipermanently attached to the vertical supporting rod. The table supporting the titration beaker has been designed to be the only easily movable part on the stand. The buret may be readily removed from its support and the electrodes may be conveniently removed and replaced.

During two years of continued use at Emeryville, five such titration stands have been found satisfactory and convenient for routine titrations. They are durable, are easy to maintain, are simple to operate, have a neat appearance and, although unitized into compact units, are sufficiently flexible for most electrometric titrations.

Apparatus

A photograph of one form of the assembly is given in Figure 1 and some details of construction are shown in Figures 2 to 4. The customary "universal" clamps have been replaced by metal connector-blocks containing holes of appropriate size and headless Bristo setscrews. No attempt has been made to use the same connector-block for all purposes. A modified thermometer clamp replaces the customary buret holder or clamp. A thick piece of polished Bakelite is used as a combination electrode support, beaker cover, and buret-tip support. The titration vessel, which is a 250-ml. tall-form electrolytic-type beaker, is supported on a Bakelite table attached to a friction clamp. The glass, propeller-type stirrer is supported and operated by a small variable-speed motor; the Eastern Engineering Company variable-speed stirrer, Model 1, has proved satisfactory. The motor and rheostat are conveniently mounted on the same support, as shown in Figure 3 (left).

Pencil- or stick-type electrodes are used throughout; whenever a salt bridge is used, contact is made with the titration medium through a ground-glass joint. The durable, permanent Beckman glass and calomel electrodes, manufactured by the National Technical Laboratories, have been found ideally suited for use in the titration assembly. For convenience, metallic electrodes—i. e., silver, platinum, and tungsten—have been mounted, as shown in Figure 2 (left), so as to be easily interchanged with the Beckman electrodes.

Figure 1. Titration Assembly Prepared for Neutralization Titrations

Beckman Model O electronic voltmeter and Beckman glass and calomel electrodes

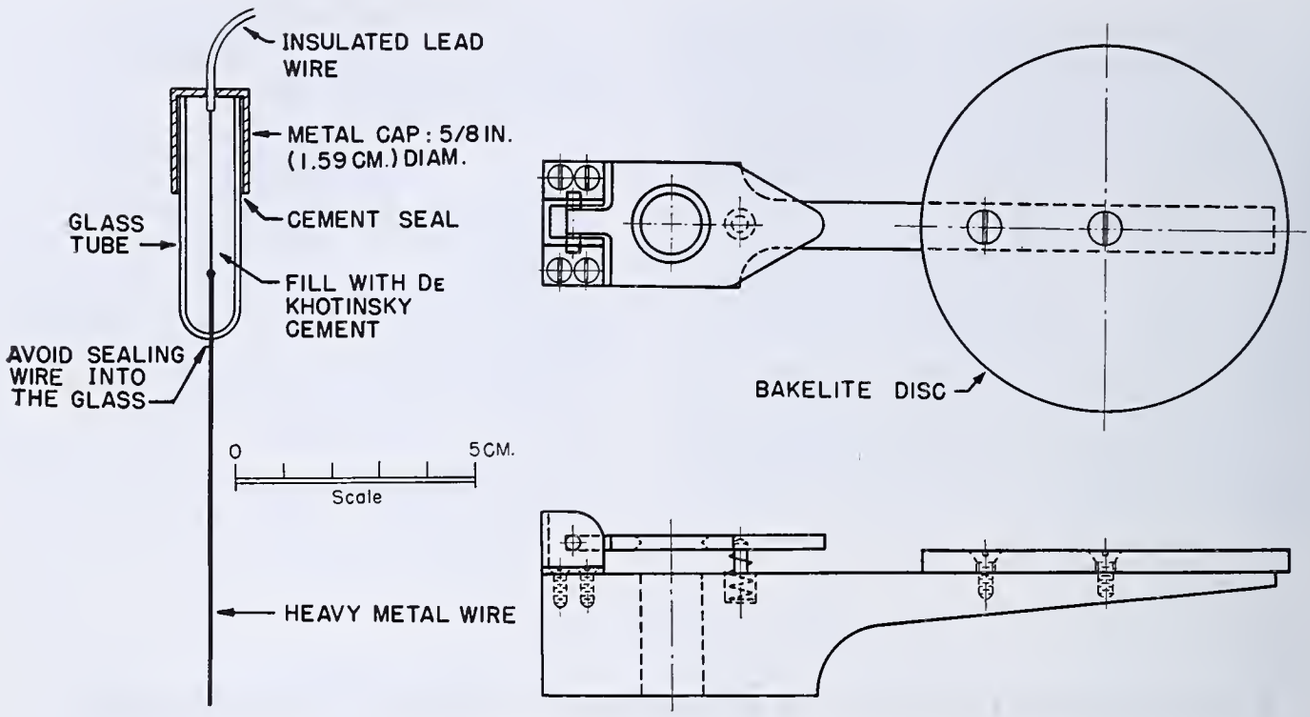

FIGURE 2. METAL ELECTRODE (*left*) AND MOVABLE BEAKER SUPPORT (*right*)

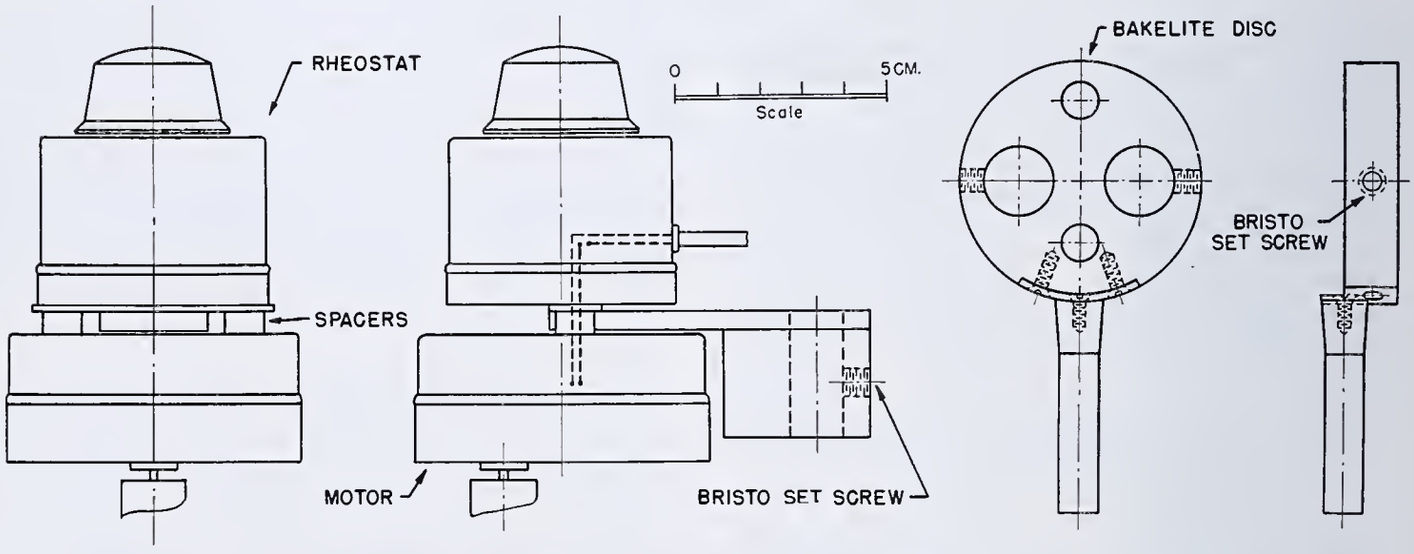

FIGURE 3. MOTOR AND RHEOSTAT MOUNTING (*left*) AND COMBINED ELECTRODE HOLDER AND BEAKER COVER (*right*)

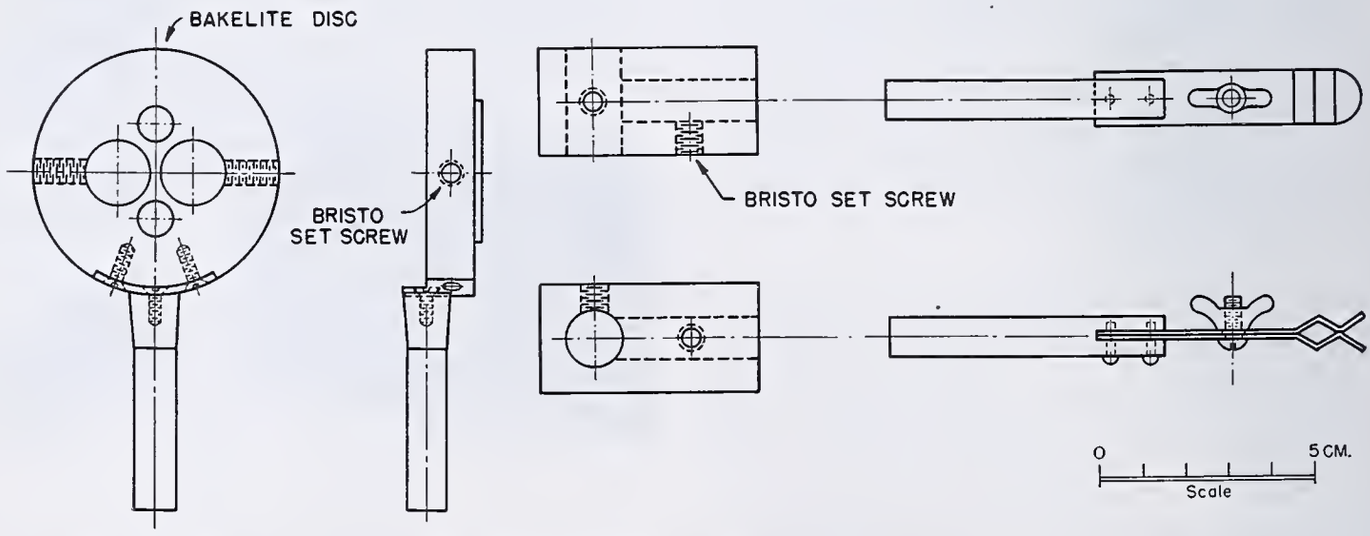

FIGURE 4. ELECTRODE SUPPORT DISK FOR SEMIMICROTITRATIONS (*left*) AND SUPPORT BLOCK AND BURET CLAMP (*right*)

The assembly is made on any 1.25-cm. (0.5-inch) ringstand rod. The various metal parts are made of brass and subsequently chromium-plated. This plating has proved satisfactory as a protection against attack by the laboratory fumes and affords a durable, neat finish.

The apparatus may be readily adapted to semimicrotitrations, of 5- to 10-ml. initial volume of solution, by changing the Bakelite electrode support disk and using a special titration cell in place of the tall-form beaker. The Bakelite disk of Figure 3 (right) is replaced by the one shown in Figure 4 (left), in which the holes are brought closer together. The modified disk has a small raised portion, 33 mm. in diameter, on the underside. The special titration cell is made from Pyrex glass tubing 34 mm. in inside diameter; it is sealed off flat at one end and is cut to give an internal length equal to 11.2 cm. The blades on the glass propeller stirrer are ground down to be 12 to 13 mm. in diameter. The regular Beckman electrodes and stirrer are adjusted so that they almost touch the bottom of the special cell when the latter is in position on the stand. The buret is made with a 15-cm. (6-inch) tip that has a 10- to 15-mm. offset about 12 cm. from the end tip.

Magnetic Stirrer for Use in the Cup Type of Moisture-Transfusion Apparatus

HORACE K. BURR AND ALFRED J. STAMM, Forest Products Laboratory, Madison, Wis.

THE cup type of moisture-transfusion apparatus is the chief means used for determining the passage of moisture under a relative humidity gradient through paper, impregnated paper, film material such as cellophane, and thin sections of wood.

Water, an aqueous solution of sulfuric acid, or a saturated salt solution in equilibrium with a specific relative humidity is poured into the bottom of a cup. The membrane material to be studied is sealed across the top of the cup. The cup is then weighed and the loss or gain in weight with time determined when the cup is exposed to a lower or higher relative humidity than that set up within the cup.

This simple technique is subject to one serious source of error, especially for relatively permeable membranes—namely, vapor is not supplied to the lower surface of the membrane as rapidly as it can pass through the membrane, and moisture is not removed from the upper surface of the membrane as rapidly as it passes through the membrane.

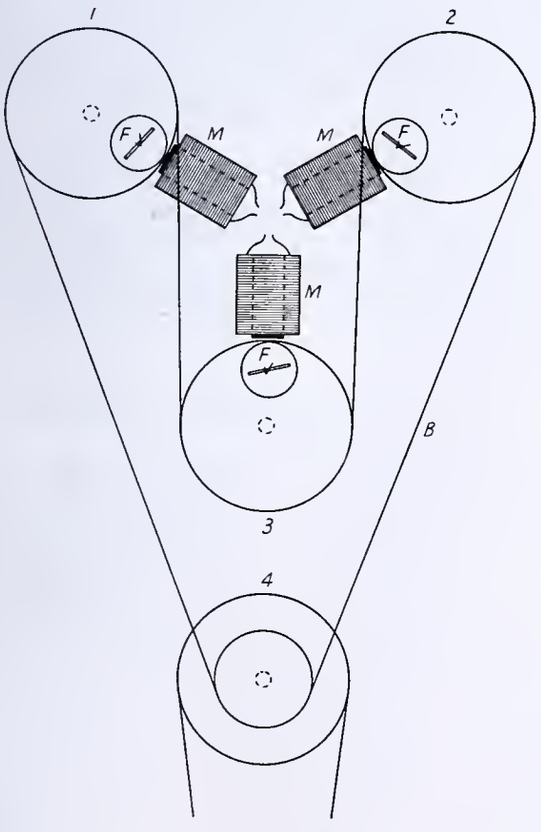

FIGURE 1. INTERNAL STIRRER FOR A CUP-TYPE MOISTURE TRANSFUSION APPARATUS

Part of the relative humidity gradient thus occurs through the air on each side of the membrane. This situation can be readily corrected on the outer surface of the membrane, when humidity rooms or chambers are used, by blowing air across the surface with an electric fan, or rapidly passing air over the surface from a humidification train.

The device shown in Figure 1 was designed to correct this same difficulty within the cup. It does not interfere with making a vapor-tight seal of the membrane. It permits easy removal of the cups for weighing and does not increase the weight of the cups sufficiently to decrease the accuracy of weighing. It consists of four horizontally mounted pulleys. Pulleys 1, 2, and 3 serve as the supports for three moisture-transfusion cups, in this case glass crystallizing dishes, which are slightly smaller in diameter than the pulleys. The cups may be clipped to the pulleys so as to be readily removable. The three pulleys are rotated by a continuous belt, *B*, by pulley 4.

Rings made by bending 18-gage sheet-iron strips 1 cm. wide into circular cylinders 3 cm. in diameter were completely covered with tinfoil on the circular surfaces as well as on the ends to make sealed drums, *F*. Small tinfoil vanes, *V*, were sealed horizontally on the top face of the drums. These drums, which served as the stirrers, weighed only 5 grams each. They were floated on the liquid in the crystallizing dishes, after which the membranes were sealed to the top. Three electromagnet coils, *M*, taken from old electric doorbells, were mounted so that the magnet surface just cleared the side of the crystallizing dish. These electromagnets were operated from the secondary of an ordinary doorbell transformer (18 volts). When the pulleys were operated at a speed of about 60 revolutions per minute, the drums, *F*, were held in a position near the electromagnets as the pulleys and crystallizing dishes rotated. Because of the friction of the drum stirrers on the side of the crystallizing dishes, they rotate about their own axes. The vane, *V*, on the drum thus rotates with respect to the base on which the apparatus is mounted and also with respect to the crystallizing dish, causing an efficient stirring of the air. The rotation of the crystallizing dish with respect to the stirring drum, *F*, also adequately caused a continual formation of a new liquid surface.

The apparatus was set up in various relative humidity rooms for making the measurements. An electric fan was directed across the membrane faces. Good check values were obtained when similar membranes were used on each of the crystallizing dishes with a liquid giving the same relative humidity inside each. When the stirring drum was left out of one of the crystallizing dishes, the rate of loss of moisture from that dish was materially less than from the other dishes. When four thicknesses of noncoated Cellophane No. 600 were used for the membrane and the relative humidity gradient was from 100 to 80 per cent, the presence of the stirrer increased the rate of moisture loss by about 70 per cent. Further increase of the rate of stirring had a negligible effect upon the rate of loss of moisture.

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Improved Apparatus for Micropreparative Work

HERBERT K. ALBER, 200 West Springfield Road, Springfield, Penna.

IT HAS been shown by the author (*1–3*) that the qualitative schemes for the identification of organic compounds developed by Mulliken (*9*) and Shriner and Fuson (*10*) operate with equal efficiency if small amounts of materials are available; systematic studies, however, were necessary in order to select the most suitable procedures for such a scheme of qualitative organic microanalysis. The first step to be taken in the process of identification is the purification of the organic compound to a degree commensurate with the stability, the amount of sample available, and the accuracies established for determination of the physical constants. The principal procedures of this preliminary step are again applied in the final step of the identification—i. e., the preparation of a characteristic derivative.

During the investigations on procedures for the purification of centigram and milligram amounts of organic substances (*2*) various kinds of apparatus have been developed or modified in order to obtain satisfactory results within a reasonably short time; three of these are described here in detail.

Improved Balance for Micropreparative Work

Standard as well as microchemical balances have been applied in the preparation of derivatives, estimation of yields, determination of solubility, semiquantitative elementary analysis (*1*, *2*), etc. A small, sturdy, portable, and inexpensive instrument which permits rapid weighing of 1-mg. samples to within 0.1 mg. is needed for this work.

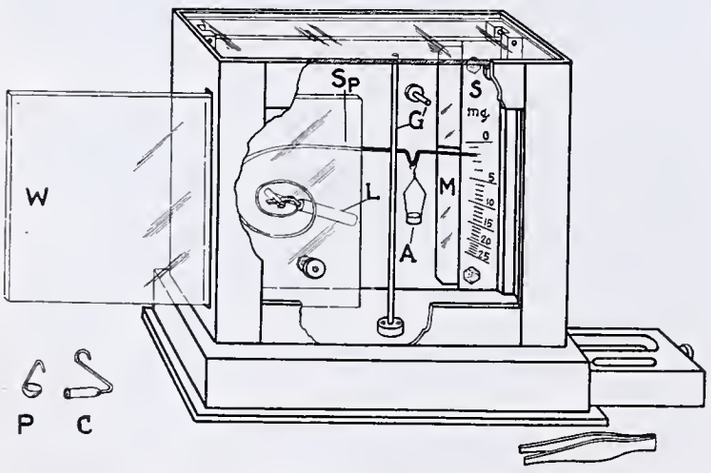

FIGURE 1. IMPROVED BALANCE FOR MICROPREPARATIVE WORK

The balance of Friedrich (*7*), a modified Emich-Salvioni balance (*6*), has been further improved. A wooden case 195 × 65 × 155 mm. (Figure 1) is mounted on a base, which contains a drawer for the weighing accessories, such as the forceps or the weighing vessels; the front window, *W*, is closed during the weighing in order to protect the spiral spring, *Sp*, from draughts. The mirror, *M*, beside the exchangeable paper scale, *S*, both of which are mounted on a glass plate, minimizes the error due to parallax when determining the position of the pointer. The aluminum-painted background facilitates the reading. Guide posts, *G*, keep *Sp* in place when the load is varied.

The weighing vessels vary in form according to the procedure in which they are used. Aluminum dish *A* serves for weighings in general preparative work. Platinum dish *P* is recommended for the ignition test and semiquantitative residue determinations (*1*, *2*), and glass capillary *C* is suitable for transferring the weighed substance into sodium fusion tubes in the semiquantitative elementary analysis (*1*, *2*).

Most weighings are made by difference; the spiral spring comes quickly to rest, and a single weighing is accomplished within a minute. The tension of spring *Sp* is adjusted by means of the lever arm, *L*, so that weighing vessels of different capacities and weights may be used. To obtain sensitivities of 0.1 mg. the empty weighing vessels, *A*, *C*, and *P*, must not be heavier than 80 mg. The values on the exchangeable paper scale, *S*, should be checked from time to time with 10-mg. weights and wires of known lower denominations.

This balance replaces more expensive preliminary balances in quantitative organic microanalysis—e. g., it is of value in the quick determination of the weight of pellets, of the solvent in the Rast-Pregl camphor method for the determination of molecular weights, etc. (The apparatus described in this paper is obtainable from the Arthur H. Thomas Co., Philadelphia, Penna.)

Mortar for Grinding and Mixing

It is often desirable to homogenize mixtures previous to taking samples for analytical work or to grind organic compounds into a homogeneous mass, in order to increase solubility (*2*), etc. Aside from the small conventional agate mortars of about 25-mm. bowl diameter, no suitable mortars are available for the efficient treatment of centigrams or milligrams of organic substances. A micromortar of Pyrex glass was described for triturating single insects with small amounts of liquids in virus studies (*4*); this instrument, however, is not applicable for grinding and mixing solids. After some attempts (*5*) a mortar has been developed which is convenient, allows rapid manipulations, and is economical of material.

The micromortar, *M* (Figure 2, *A*), is made of heavy chemical porcelain and is glazed, with the exception of the roughened outside base of 50-mm. diameter which assures a firm support on the

table during grinding operations. The inside has the form of an inverted cone 15 mm. in length, the opening being 20 mm. wide, narrowing to 7 mm. To prevent loss of material the lip is eliminated.

The unglazed base of the pestle, P, is 6 mm. wide. The radius of curvature of the bottom part of the mortar, r_m (Figure 2, B), is designed to ensure close contact between the material and the acting surfaces of both mortar and pestle; a pestle with a base of a slightly smaller radius of curvature, r_p, than that of the mortar is used.

If freedom from small contaminations is of vital importance or hard substances are to be ground, as in mineral analysis, the recently introduced sillimanite (Mullite, Coors, U. S. A.) is recommended in preference to porcelain.

The efficiency of the micromortar was tested in two ways: Weighed amounts of substances of varying hardness were ground in the mortar to a fine powder for about 5 minutes. The powder was removed with a microspatula and weighed again on an ordinary balance. A few results are reported in Table I.

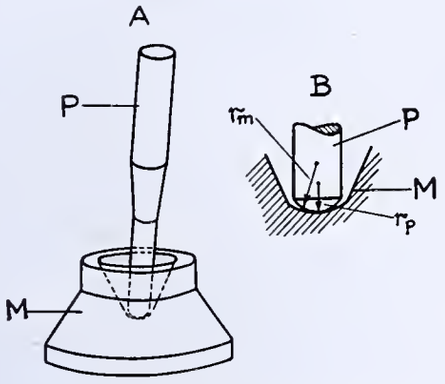

Figure 2. Micromortar with Pestle (A) and Ratio of Curvatures of Pestle and Mortar (B)

Low recoveries of 30 and 50 per cent were observed on exceedingly soft, hygroscopic, and highly charged (finely powdered) compounds. The high recoveries of sea sand in Table I are characteristic of materials with a hardness above 6 in the Mohr scale, since they become contaminated with material removed from the surface of the mortar or pestle. Sea sand ground in a sillimanite micromortar as above gave recoveries of 95, 93, and 93 per cent of 25.0, 18.1, and 10.5 mg. used.

Table I. Recovery of Material on Grinding in Micromortar

Substance	Taken (Original)	Recovered (Powdered)	Recovery
	Mg.	*Mg.*	%
Benzoic acid	50	44	88
	20	17	85
	10.0	6.4	64
	5.0	2.6	52
KH_2PO_4	50	42	84
	20	17	85
	10.0	8.0	80
	5.0	4.0	80
Sea sand	50	48	96
	30	28	93
	11.4	11.6	102

The efficient mixing of heterogeneous substances in the micromortar was tested on a two-component system, the ratios being varied from 100:1 to 1:100. After 3 minutes' grinding and mixing, 2 aliquot portions of approximately one third each of the total original weight were taken, and a quantitative determination was made of one compound, or of a characteristic element in this compound. Potassium dihydrogen phosphate and sodium oxalate were selected for the experiments in Table II, because they had given approximately identical recoveries in grinding experiments. The degree of uniformity of the final product was determined by analyzing each aliquot portion for phosphorus, using the colorimetric procedure of King (*8*).

Table II. Efficiency of Mixing Two Solid Compounds in Micromortar

Components of Mixture Weighed Out KH_2PO_4	Components of Mixture Weighed Out $Na_2C_2O_4$	Approximate Ratio	Deviation of P Found from P Calculated in Aliquot Portions	
Mg.	*Mg.*		%	%
3.0	100	1:100	−2.8	−9.5
9.3	99	1:10	+0.7	+2.3
50.0	50.0	1:1	−0.4	+1.8
100	10.0	10:1	+0.7	−1.4
100	1.5	100:1	+0.3	−0.4

Appreciable errors were noticed only when very small amounts of the component to be analyzed were ground with large amounts of the admixed material, ratio 1 to 100. The results on the ratio 100 to 1 may be taken as an indication of the accuracy and precision of the phosphorus determination.

Combined Separatory and Sedimentation Funnel

Many procedures for separation, isolation, or purification of organic constituents involve extraction; a study of continuous extractors for solids has been reported recently (*3*). A special problem is the separation of a mixture of several liquids, in which the components are isolated by consecutive washings with several portions each of water, alkali, acid, and organic solvents. After extraction or washing a careful separation of the layers becomes a difficult task in an ordinary funnel, especially if a precipitate is formed during the process. The upper layer should not be "poured out" of the top, as this leads to unavoidable loss of material. The treatment of a solid compound in successive steps with different organic solvents—e. g., in the determination of solubility—is also difficult without a special separatory funnel.

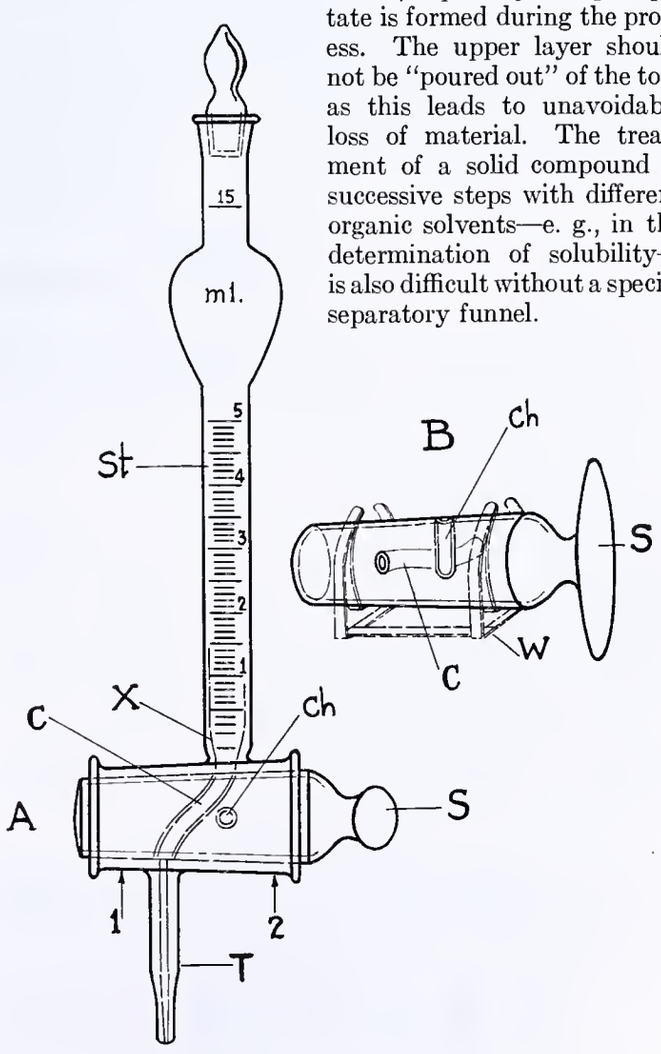

Figure 3. Combined Separatory and Sedimentation Funnel

The combined separatory and sedimentation funnel, Figure 3, is a useful apparatus for the above-mentioned procedures. The dimensions are best chosen according to the problem; the ones given here are recommended for milligram and centigram amounts of solids, and milliliters or less of liquids. The calibration on the cylindrical stem, *St*, permits the estimation of used volumes and volume changes. *St* has an inner diameter of from 7 to 8 mm., and is graduated to 5 ml., each milliliter being subdivided into 0.1 ml.; it extends into a bulb of 10-ml. capacity, which is closed with a ground-glass stopper, the total capacity of 15 ml. being indicated by a mark on the upper neck. (Funnels have been used with stems graduated to 10 ml. and extending into a bulb giving a capacity of 25 ml.) The lower end of the stem is gradually reduced, at *X*, within a length of from 15 to 20 mm. to 3 to 4 mm. inner diameter, thus forming a smooth cone.

The hollow stopcock, *S*, with an average diameter of about 20 mm. is equipped with a bent capillary, *C*, 3 to 4 mm. in diameter, leading to the tip, *T*, 15 to 20 mm. long (Figure 3, *A*). With stopcock *S* (Figure 3, *B*) turned 90°, the opening of the stem coincides with that of the cylindrical sediment collector, *Ch*, 3 to 4 mm. in inner diameter and 10 to 12 mm. long, having a capacity of 70 to 150 cu. mm. The size of *S* may seem excessive for the small amounts of material handled, but this is necessary to prevent leakage, as in most cases no grease is used between positions 1 and 2 (Figure 3, *A*). Grease-collecting grooves in positions 1 and 2 were tried, but they merely complicated the apparatus.

The technique of manipulating this separatory funnel is simple. If the liquid with higher density is in the range of about 100 cu. mm., it is collected in the sediment collector, *Ch*, by settling; the stopcock is turned into position *A*, and the lighter liquid is removed through capillary *C* and tip *T* without disturbing the liquid in *Ch*. After turning back the stopcock into position *B*, the treatment is continued with a fresh portion of solvent. Finally, stopcock *S* is pulled out from the separatory funnel and placed in a small wire rack, *W* (Figure 3, *B*); the liquid is removed from the chamber by means of a fine capillary pipet.

The same procedure is applied for solid sediments, which are dried in the sediment collector, weighed, and removed with a microspatula or dissolved in a suitable solvent.

The efficiency of this separatory funnel has been tested on several separations of unknown mixtures of two to four liquids, with experiments on liquid-liquid extraction using water, ethyl alcohol, ethyl ether, amyl alcohol, petroleum ether, carbon tetrachloride, benzene, etc., with processes necessitating a salting-out with sodium chloride, magnesium sulfate, or potassium carbonate, and with percolation experiments on fibrous materials (drugs) packed in the stem, etc.

Emulsions which are difficult to break up usually collect in a thin layer on the interface of two immiscible liquids. After the lower layer has been carefully drained through the capillary, the emulsion in some instances settles out overnight in the sediment collector and can easily be removed.

Acknowledgment

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A Measuring Microscope

Technique for Measuring Vertical Distances up to Several Centimeters with a Precision of 0.00005 Cm.

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In some investigations in this laboratory it became imperative, because of the accuracy desired, to use a cathetometer which was accurate to 0.0001 cm. and had a working range of at least 10 cm. Since such an instrument was not available, the need was supplied by constructing the measuring microscope described here, which has a range of 16 cm. and can be read to 0.000058 cm. The difficulties that were encountered and solved in constructing and calibrating the instrument to this precision should be of some general interest.

Construction

The general construction of the instrument is evident from Figure 1. The cross feed from a small lathe was attached rigidly to a heavy tripod base and the barrel and focusing arrangement of an ordinary microscope were attached by means of a heavy angle iron, *A*, bolted to the slide. The sliding ways were carefully lapped with fine emery with strokes running the full length of the ways. The slide was frequently reversed during the grinding to remove any bow which might exist. Grinding was continued until the slide could be adjusted to a snug fit with light even drag over the entire range of motion. The lead nut attached to the slide and the lead screw were carefully machined with a square thread, 12 turns to the inch. The lead screw was fitted by means of a tapered pin with a steel collar by which the weight of the slide was carried on the adjustable brass bearing, *B*. The position of *B* was adjusted so that the lead screw lined up perfectly with the direction of motion of the slide and there was no tendency to bind at any point. A pointer on the slide permitted reading the number of turns of the lead screw on a fixed scale, and a graduated disk on the lead screw and vernier read to 0.1° of arc.

Lengths could be read with a precision of 0.001 cm. merely by taking one turn of the lead screw equivalent to 0.21 cm. (0.083 inch). However, in order to obtain the next significant figure, further calibration and a consistent technique were required.

Testing and Leveling the Instrument

A 40-gram weight was suspended by means of a 1.5-mil nickel wire in water to minimize vibrations. The microscope was focused on the wire and leveled by means of the leveling screws until the wire remained in focus, and the vertical cross hair of the eyepiece did not deviate more than half the width of the wire, while traveling over the range of the instrument. This insured a vertical alignment with a deviation of less than 0.025 mm. (0.001 inch) and also showed that the sliding ways were not warped appreciably at any point. Since the two cross hairs in the eyepiece were perpendicular, alignment of the vertical cross hair parallel to the wire served to make the other cross hair horizontal.

Reading Technique

Because of the weight of the slide it was necessary to use a heavy oil on the lead screw to prevent wear. Many different grades of lubricant were tried. With temperatures from 23° to 27° C., the best results were obtained with medium heavy cup grease on the slide and S. A. E. 120 lubricant applied freely to the lead screw. This gave a film which had a thickness much greater than the limit to which the microscope was being read. It was found that the thickness of the oil film could be kept constant and the reading of a given fixed point could be duplicated to 0.1° by means of the following technique: Flood the screw with oil while the slide is at the bottom of the screw, run the slide to the top, apply more oil to the screw, run slide back to the lowest point on the instrument, and raise to the point being read. It was very important in the process of taking a reading that the cross hair did not go past the index so that the motion of the slide had to be reversed. Using this technique, any error greater than 0.1° in measuring length results from imperfections in the lead screw.

Imperfections in Lead Screw

Periodic Errors. Periodic errors are imperfections in the screw which are repeated monotonously at corresponding points on successive turns. These variations can be successfully eliminated by taking readings on the given length at four points 90° apart and averaging the results. This uniform displacement of the length was accomplished by moving the cross hairs in the micrometer eyepiece.

Longitudinal Variations. Longitudinal variation in the lead screw was calibrated in terms of a fixed length which was given an arbitrary value slightly less than its measured length, so that the deviations would be small positive numbers.

In calibrating, the fixed length was measured from a turn near the top of the screw which was taken as the reference turn. The measured length less the length arbitrarily assigned was taken as a correction corresponding to the turn in which the lower reading was taken. The standard length was then lowered by successive steps each equal to its length; cumulative deviation was plotted against the turn number corresponding to the lower reading. A smooth curve drawn through these points was a first approximation of the calibration.

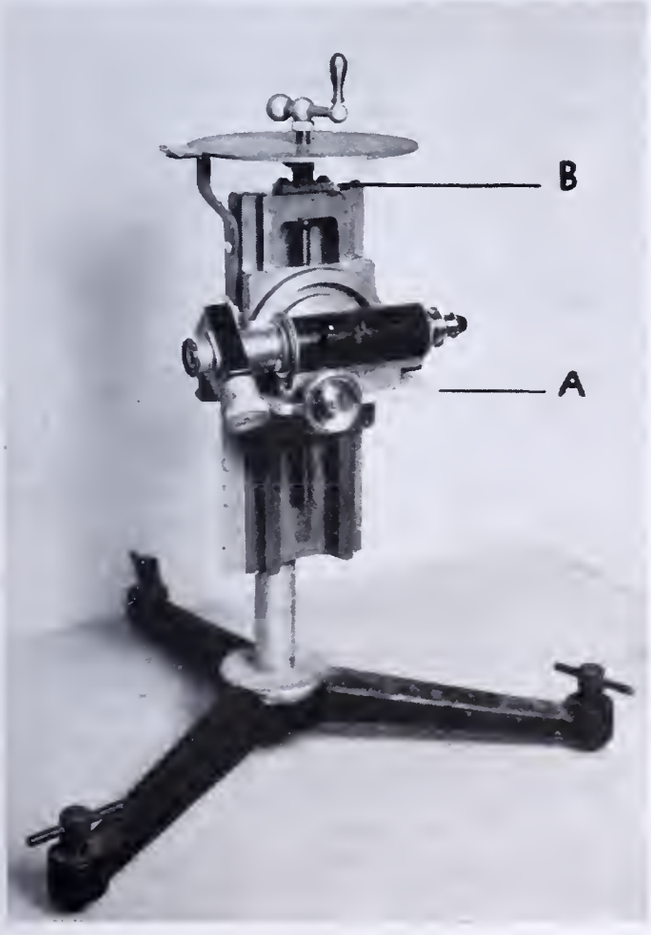

Figure 1. Measuring Microscope

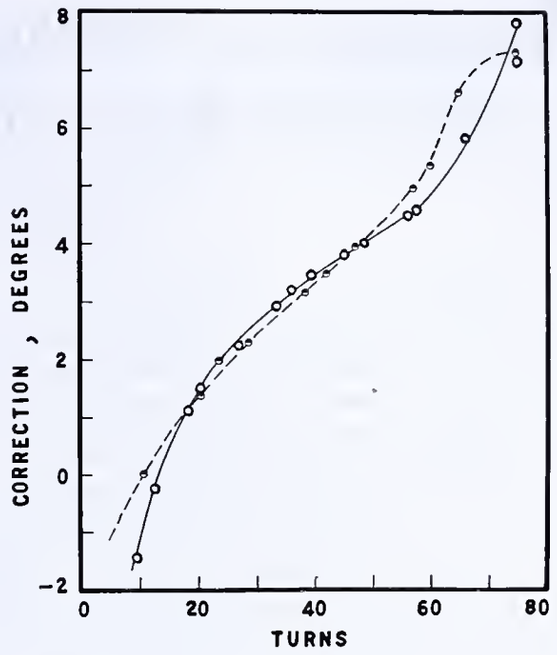

Figure 2. Longitudinal Calibration Curves

From measurements with shorter lengths it was found that the central portion of the screw was uniform. This corresponds to a linear portion of the calibration curve as it was plotted. In this range other reference points may be accurately established and the deviation at other points on the curve outside this range determined by further measurements with the standard length. Figure 2 shows a calibration curve obtained in this way, where the solid curve is the original curve and the dotted curve is a calibration obtained with a different standard length after 2 years of use. It is evident from a comparison of the two calibration curves that the instrument is improving with use.

Although the lead screw was cut on a new precision lathe at 12 turns to the inch, the screw was further calibrated:

Two indices, approximately 6.5 cm. apart (approximately the uniform center range of the microscope) were made on a small quartz fiber and the exact distance was measured with the instrument described and compared to the length obtained, using a measuring microscope which read directly to 0.001 cm. by means of a vernier and scale. The readings were made in a thermostat at 25° C. with a small weight attached to the fiber to give it vertical alignment. A ratio of the two readings (uncorrected) gave a conversion factor, $1° \equiv 0.0005879(8)$ cm. This agrees well with the conversion factor obtained by assuming 12 turns to the inch, $1° \equiv 2.540005/12 \times 360 = 0.00058796$ cm.

The instrument therefore permits the measurement of relative lengths with a high degree of precision, the absolute accuracy depending also on the accuracy with which the length of the standard is known.

Precision

The validity of the calibration and technique was tested by measuring different fixed lengths at different points on the scale. Each reading was corrected by subtracting the correction taken from the calibration curve, and the corresponding length, which was the difference between the corrected readings, was corrected for the thermal expansion of the lead screw when necessary. Using 25° C. as standard, this correction for thermal expansion amounted to 0.0001 cm. for a length of 10 cm. and a temperature difference of 1°. The lengths thus corrected could be duplicated to 0.1° in the middle range of the instrument and to 0.2–0.3° near the ends of the range. When possible the extreme ends of the range were avoided in actual use.

Removal of Nitrogen Oxides in Semimicro-determination of Carbon and Hydrogen

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In recent years the unsuitability of lead dioxide for use as an absorbent for nitrogen oxides in organic combustion analysis has been repeatedly demonstrated. Among the suggested substitutes for lead dioxide have been Hopcalite (Corwin) and *p*-aminoazobenzene (Dombrowski) which are placed between the Anhydrone and Ascarite tubes. Solutions of strong inorganic oxidizing agents in concentrated sulfuric acid—e. g., 0.02 *M* potassium permanganate in sulfuric acid—will absorb nitrogen oxides but not carbon dioxide. Nitrogenous organic compounds have been satisfactorily analyzed, using sulfuric acid solutions of potassium permanganate in a special absorption vessel following the Anhydrone tube.

In a search for a substitute for lead dioxide that could be used either in the combustion tube or in the absorption train various metals and metallic oxides have been tested for their ability to decompose or absorb nitrogen dioxide from mixtures of nitrogen dioxide and air.

ONE of the serious sources of error in present methods for the determination of carbon and hydrogen is the use of lead dioxide to remove the nitrogen oxides from the products resulting from the combustion of organic compounds containing nitrogen (*2, 6, 15, 25, 26, 41, 42, 43*). These oxides must be removed prior to the absorption of carbon dioxide, since their acidic nature will cause their absorption by the alkaline reagents used for absorbing the carbon dioxide and consequently will lead to high results for carbon. Various absorbents, which will not react with the nitrogen oxides in the gas stream of combustion products, may be used for the water formed. These nitrogen oxides can be removed by absorption by a suitable substance or reduction to free nitrogen.

Lead Dioxide and Proposed Substitutes

Lead dioxide, long used in macroanalysis (*13, 20*), was retained in his microanalytical procedure by Pregl, who called it "the most critical reagent" in the method (*39*). The use of lead dioxide in both micro- and macroanalysis has been studied critically by numerous investigators (*2, 6, 7, 9, 15, 20, 21, 24, 41, 42, 43*). The mechanism of the absorption of nitrogen oxides by the lead dioxide was investigated by Kirner (*19*) who summarized previous work on this topic. Specific sources of error (*3, 14, 23, 25, 26, 27, 30, 39*) are the tendency of lead dioxide to absorb carbon dioxide when dry and to give it off again in the presence of small amounts of water or of the combustion products of organic compounds containing nitrogen, halogen, or sulfur, and the gradual absorption and elimination of water from the lead dioxide. Boëtius (*4*) and Niederl and Whitman (*35*) consider the time and amount of gas specified by Pregl for the purpose of sweeping out the combustion gases insufficient for the complete removal of the water from the lead dioxide. These sources of error are more or less eliminated by keeping the lead dioxide at a temperature of 180° to 200° C., in which range the vapor pressure of water–lead dioxide system has a constant value, which is high enough to minimize the absorption of carbon dioxide and low enough to guard against storing up appreciable amounts of water (*22, 39*).

The various absorbents and reagents for the removal of the oxides of nitrogen suggested in the literature have been tested by Corwin (*6*) and their advantages and disadvantages studied. Most of the substitutes proposed for lead dioxide have been metals intended to reduce the oxides of nitrogen to elementary nitrogen. Müller and Barck (*29*) studied the decomposition of nitric oxide when heated with various metals. The most efficient reducing agent for the oxides of nitrogen seems to be metallic copper, first used by Gay-Lussac (*17*). The use of copper in combustions taking place in oxygen, however, necessitates the introduction of a freshly reduced copper spiral for each determination. The latter was avoided by Benedict (*1*) who burned a weighed sample of high carbon content with each nitrogen-containing sample, the combustion of the former producing the freshly reduced copper surface needed to reduce any oxides of nitrogen formed by the second sample. A more satisfactory solution of the problem involving the use of metallic copper was proposed by Niederl and Whitman (*34*) who burned the sample in an atmosphere of nitrogen, using the combustion tube filling recommended by Pregl (*38*) for the micro-Dumas method. The oxygen for the combustion was supplied by copper oxide mixed with the sample. Lindner (*24*) also used a combustion tube containing copper spirals, employing nitrogen as the transport gas and oxygen or air in the actual combustion. After four or five runs Lindner's copper spirals had to be renewed. Pregl (*37*) claims that metallic silver, suggested by Dennstedt (*8*) and Dubský (*11*), is incapable of decomposing the higher nitrogen oxides. Untreated Raney nickel-aluminum catalyst, kept at 200° to 250° C., has been found by Gardella and Baldeschwieler (*16*) to be effective in removing nitrogen oxides in macroanalysis.

Perkin (*36*) suggested replacing the lead dioxide used in macroanalysis with potassium chromate. Corwin (*6*) found that dry Hopcalite could be substituted for lead dioxide as an absorbent for the oxides of nitrogen. The Hopcalite, maintained at a temperature of 125° to 175° C. and placed between the Anhydrone and Ascarite tubes, apparently has to be regenerated after each run by heating to 300° C. The analytical method for carbon and hydrogen based on the use of Hopcalite was claimed by Corwin to be superior in accuracy to any micromethod for carbon and hydrogen previously described in the literature. In 1940 Dombrowski (*10*) also proposed an absorbent for the nitrogen oxides inserted between the tubes containing the absorbents for the water and the carbon dioxide. The adsorbent used was *p*-aminoazobenzene, which was moistened with a saturated solution of boric acid and potassium dichromate and was placed in a special U-shaped tube, followed by a layer of magnesium perchlorate contained in the same U-shaped tube. This filling had to be renewed after five or six analyses. Dombrowski's results for hydrogen on five nitrogen-containing compounds, each run in duplicate, using magnesium perchlorate as water absorbent, were high, on an average, by 0.23 per cent, indicating that the magnesium perchlorate did not absorb the oxides of nitrogen to any appreciable extent. This latter fact was confirmed by the experimental results described below. Corwin (*6*) claims, however, that Anhydrone absorbs nitrogen dioxide but not nitric oxide. The absorption of 1 per cent of the nitrogen in a sample as nitrogen dioxide by the Anhydrone would cause an apparent increase of 0.37 per cent in the hydrogen content.

Purpose of Investigation

The purpose of the investigation reported in this article was twofold. First, a simple absorbent for the oxides of nitrogen was sought which could be used outside the combustion tube; this would avoid the use of lead dioxide and would permit the use of a simplified combustion tube packing. Secondly, possible substitutes for lead dioxide were investigated for use in the combustion tube or in the absorption train. For the former purpose, various solutions were tried as absorbents for nitrogen oxides; for the latter purpose, various metals and metallic oxides were tested for their power to remove nitrogen oxides from a stream of gas.

Preparation of Mixtures of Oxides of Nitrogen and Oxygen

In anticipation of testing the absorptive capacity of many materials for oxides of nitrogen, a permanent setup was constructed for generating nitric oxide, measuring a definite volume, and mixing with oxygen in any desired proportions. The apparatus, shown in Figure 1, is an adaptation of the nitric oxide generator described by Kirner (*19*), although a different reaction was used.

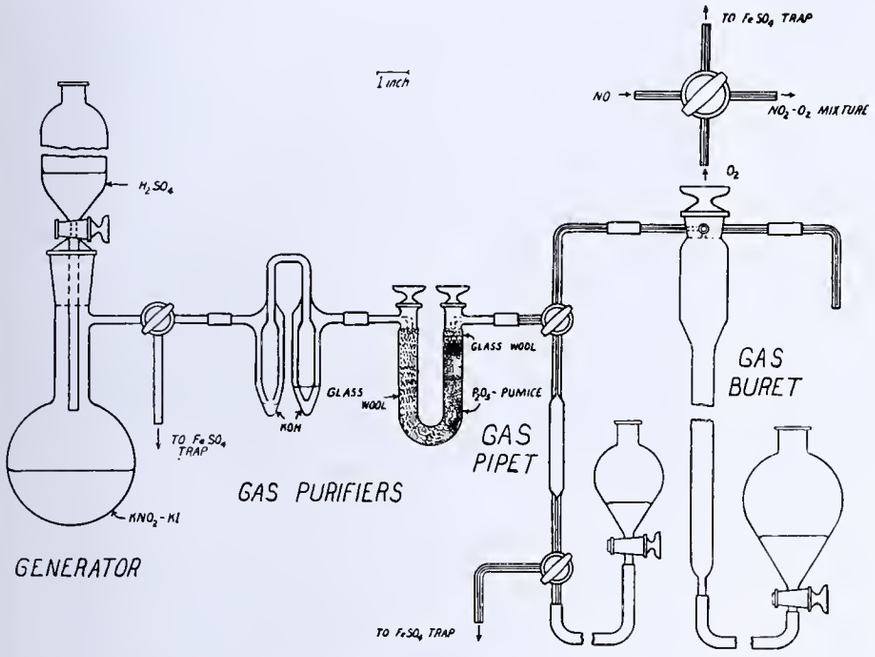

FIGURE 1. APPARATUS FOR PREPARATION OF NITROGEN DIOXIDE–OXYGEN MIXTURES

Nitric oxide was made in the generator by the action of dilute sulfuric acid on a 1 to 1 molar solution of potassium nitrite and potassium iodide (*44*). The nitric oxide was washed and dried by passage through saturated potassium hydroxide solution and through phosphorus pentoxide mixed with pumice. Small volumes of nitric oxide were measured out in the calibrated gas pipet (volume = 2.5 cc.) while large volumes of nitric oxide were measured out directly in the gas buret (volume of the upper bulb = 50 cc., volume of the lower calibrated tube = 50 cc.), where the samples of nitric oxide were diluted with oxygen and from which the gas mixture was led to the reagent being tested for removal of nitrogen oxides. Before using, the apparatus was cleared of air by flushing with nitric oxide. The samples of nitric oxide taken were absolutely colorless. On the addition of oxygen brown nitrogen dioxide was formed. Mercury was used as confining liquid in the gas pipet and gas buret, and ferrous sulfate solutions were used in the traps designed to keep the nitric oxide from getting into the air. The generator and purification train were kept filled with nitric oxide when the apparatus was not being used.

Under the condition of the experiments, using an excess of oxygen, the nitric oxide would be changed completely to nitrogen dioxide in the gas buret. As has been pointed out (*18, 19, 28, 40*) nitrogen dioxide and nitrogen are the probable end products from a properly conducted combustion of a nitrogen-containing compound.

Absorption of Nitrogen Dioxide by Solutions

For the experiments involving the use of an absorbing solution, absorption vessels of various shapes and sizes were tested.

Each type was placed in the combustion train, as it would be in practice, to determine how much resistance it offered to the flow of gas. Those possessing too much resistance for operation were discarded. A capillary bubbler seemed best suited to the need. Capillaries of various diameters were tested to determine the extent of foaming and the pressure necessary to produce a satisfactory flow of gas. An all-glass vessel, shown in Figure 2, was finally designed, having two bubblers, each of 1-mm. orifice, in tandem. This absorption vessel is readily filled and emptied through the stopcock. The volume of liquid usually present was about 10 ml.

On the basis of the familiar use of sulfuric acid for absorbing nitrogen oxides in the chamber process for the manufacture of sulfuric acid and of the use of potassium dichromate solution as an absorbent for the oxides of sulfur in the analytical method for the determination of carbon in steel, sulfuric acid and solutions of inorganic oxidizing agents in sulfuric acid were selected for study as absorbents for the oxides of nitrogen.

In the experiments described the mixture of nitrogen dioxide and oxygen was led from the gas buret connected to the nitric oxide generator through a capillary glass tube to the absorption vessel described, then through a bubbler into a Mariotte bottle. The bubbler contained an indicator solution of diphenylamine which gives a blue coloration with nitrogen dioxide. By this device the failure of the absorbing solution to remove nitrogen dioxide from the gas stream could be instantly detected. A blue color appeared on passing 0.01 mg. of nitrogen dioxide directly into the diphenylamine solution.

Concentrated sulfuric acid was unsatisfactory as an absorbent for nitrogen dioxide. A saturated solution of potassium dichromate in concentrated sulfuric acid was found to be satisfactory. When 1500 cc. of a 50 per cent nitrogen dioxide mixture were passed through at the rate of 5 cc. per minute, no nitrogen dioxide was detected in the scrubbed gas. To determine whether or not this absorbent would also absorb carbon dioxide, the absorption tube was placed in the combustion train between the Anhydrone and Ascarite tubes and analyses were made on standard substances. The results for carbon and hydrogen were satisfactory. Suitable experiments showed that nitrogen dioxide is either not absorbed or only negligibly absorbed by Anhydrone under the usual conditions of gas flow and nitrogen oxide concentration.

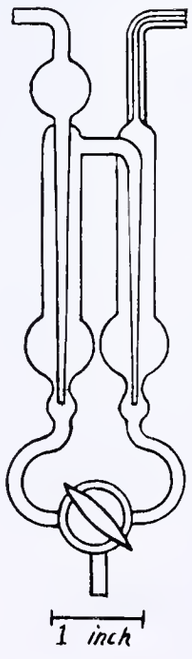

FIGURE 2. ABSORPTION VESSEL

By similar experiments sulfuric acid solutions, 0.02 *M* in potassium permanganate, 0.1 *M* in potassium persulfate, or saturated with ceric sulfate, were each found to give satisfactory removal of nitrogen dioxide from nitrogen dioxide–oxygen mixtures, ranging in concentration to 50 per cent nitrogen dioxide and in rate of flow to 10 cc. per minute. As a general procedure, it is safe to assert that any nonvolatile oxidizing agent in sulfuric acid which is relatively stronger as an oxidizing agent than nitric acid can be used to remove nitrogen dioxide from the gas stream.

TABLE I. DETERMINATION OF CARBON AND HYDROGEN

Compound	Absorbent Used	Carbon Calculated %	Carbon Found %	Hydrogen Calculated %	Hydrogen Found %
Benzoic acid	Potassium dichromate[a]	68.88	68.83	4.95	5.11
	Potassium permanganate[b]		69.06		4.97
d-Mannitol	Potassium dichromate[a]	39.55	39.58	7.74	7.83
Glycine	Potassium dichromate[a]	32.00	32.19	6.71	6.81
	Potassium permanganate[b]		31.95 32.13		7.07 7.00
p-Nitrotoluene	Potassium dichromate[a]	61.31	61.59	5.14	5.14
	Potassium permanganate[b]		61.15		5.23
3,5-Dinitrobenzoic acid	Potassium dichromate[a]	39.63	39.53	1.90	1.97
	Potassium permanganate[b]		39.70		1.95
Thiourea	Potassium permanganate[b]	15.78	15.87	5.29	5.36
p-Nitrobromobenzene	Potassium permanganate[b]	35.67	35.30 35.61	1.99	2.03 2.31
Ephedrine hydrochloride	Potassium permanganate[b]	59.55	59.50 59.38	7.99	8.18 8.15
Research substances					
$C_5H_{20}O_4N_2$	Potassium permanganate[b]	37.04	37.07 36.80	6.21	6.54 6.58
$C_7H_{13}O_3N$	Potassium permanganate[b]	52.81	52.75 52.98 52.86	8.23	8.28 8.51 8.58
$C_7H_{14}O_4N_2$	Potassium permanganate[b]	44.20	44.36 44.37	7.42	7.43 7.81

[a] Saturated solution in concentrated sulfuric acid.
[b] 0.02 *M* solution in concentrated sulfuric acid.

Analysis, Using the Proposed Absorbent

Various nitrogen-containing compounds were analyzed, using either a saturated solution of potassium dichromate in concentrated sulfuric acid or a 0.02 *M* solution of potassium permanganate in concentrated sulfuric acid to remove the nitrogen oxides from the combustion products.

The analyses were carried out on a semimicro scale, using 10- to 20-mg. samples. A combustion apparatus of the conventional type for the determination of carbon and hydrogen described by Niederl and Niederl (*31*) was used with the simplified combustion tube filling, consisting only of platinum gauze, silver wool, and copper oxide (*32*). The combustion procedure was somewhat modified because of the larger samples (*33*). All weighings were made on a Becker No. 360 semimicrobalance which could be read to 0.01 mg. Many analyses were made on standard materials such as benzoic acid and *d*-mannitol, in order to check the accuracy of using this semimicrotechnique. When this was accomplished, experimental work was started.

The absorption vessel was placed between the two absorption tubes containing Anhydrone and Ascarite. In the analyses in which the potassium permanganate–sulfuric acid solution was used, an absorption tube containing Anhydrone similar to the water-absorption tube was inserted between the absorption vessel and the Ascarite tube. A single 10-ml. filling of permanganate–sulfuric acid solution will absorb nitrogen dioxide equivalent to 0.07 gram of nitrogen and should suffice for at least fifteen analyses. The analyses are shown in Table I. In the analyses of samples containing halogen or sulfur, a 6-cm. layer of lead chromate was inserted before the copper oxide in the combustion tube, except that for the analysis of the ephedrine hydrochloride the lead chromate was mixed with the copper oxide.

Simultaneous Determination of Carbon, Hydrogen, and Nitrogen

It seemed probable that the nitrogen oxides absorbed in the sulfuric acid containing a strong oxidizing agent would be converted to nitric acid. If this were true, and if all the nitrogen in the sample were burned to oxide, then it remained only to determine the nitric acid in the absorption vessel to have a quantitative method for the determination of nitrogen simultaneously with carbon and hydrogen. With this in mind a method was devised for the determination of nitric acid in concentrated sulfuric acid which would be applicable to solutions containing permanganate, since permanganate was the oxidizing agent most readily destroyed without destruction of the nitric acid. The method of Bowman and Scott (*5*) for the determination of nitric acid in oleum and in mixed acids was adapted for use on a semimicro scale.

The potassium permanganate–sulfuric acid solution containing the nitric acid resulting from the combustion of a sample was drained from the absorption vessel into a 50-ml. beaker, in which the washings of the absorption vessel were also collected. The resulting solution was then titrated after the reduction of the permanganate. In all cases the nitrogen content found was much lower than the correct value, the nitrogen values being 1 per cent or more apart for any one sample. The error is probably due to pyrolysis of the sample before actual combustion occurs, forming free nitrogen which escapes absorption. Future work will involve study of a catalyst or method which will convert all the nitrogen in the compound to nitrogen oxides, thereby permitting the simultaneous determination of carbon, hydrogen, and nitrogen. Probably nitrogen present as nitro nitrogen can be liberated as oxide under suitable conditions.

TABLE II. REMOVAL OF NITROGEN DIOXIDE

	Temperature ° C.	Nitrogen Dioxide Concentration %	Rate of flow *Cc. per min.*	Result
		By Metals		
Aluminum	180	5	5	Partially effective
Antimony	300	2	5	Unsatisfactory
Bismuth	Below melting point	2	4	Unsatisfactory
Nickel	180	5	3	Partially effective
Silicon	300	2	1	Unsatisfactory
Silver	300	2	3	Partially effective
Tellurium	300	2	5	Unsatisfactory
Tin	180	2	5	Unsatisfactory
Zinc	180	2	5	Unsatisfactory
		By Metallic Oxides		
Cerium dioxide	300	2 to 50	1.5	Complete
Lead dioxide	180	2	5	Incomplete
Manganese dioxide	180	2 to 50	5	Complete
Thorium dioxide	180	5 to 10	5	Complete
Zirconium dioxide	180	5	5	Complete
	180	10	5	Partially effective
	"Red heat"	5 to 20	5	Complete

Removal of Nitrogen Dioxide by Metals and Metallic Oxides

In studying the effectiveness of various metals and metallic oxides in removing the nitrogen dioxide from a mixture of nitrogen dioxide and oxygen, the gas mixture was passed through a 15-cm. tube, cut from the tapered end of a combustion tube, which contained the substance under study, then through a bubbler containing diphenylamine indicator solution, and into a Mariotte bottle. The piece of combustion tube was held in a glass heating mortar, so that by use of *p*-cymene or mineral oil the substance being tested could be held at a temperature of 180° or 300° C. A thermometer was placed in the heating mortar containing the mineral oil. In some experiments, the heating mortar was removed and the tube was heated directly by a Bunsen burner and wing tip. The substance being tested was packed in a 6-cm. layer in the tube, supported at either end of the layer by asbestos plugs. In some cases the substance had asbestos plugs inserted in it at 2-cm. intervals to prevent channeling.

The conditions used and the results obtained are summarized in Table II. An unsatisfactory result indicates that the substance in the state tested failed to remove the nitrogen dioxide from the gas stream at the concentration of nitrogen dioxide, rate of flow, and temperature tried. Failure to remove nitrogen dioxide was indicated by the appearance of a blue color in the diphenylamine solution.

METALS. Aluminum powder or nickel powder failed to remove the nitrogen dioxide completely from mixtures of nitrogen dioxide and oxygen. Mixtures of aluminum and nickel powders in the proportions 1:3, 1:1, and 3:1 were no more satisfactory at 180° or 300° C. A coil of silver wire and a silver-asbestos contact mass were found to be only partially effective under the conditions tried. The specimens used in the other experiments listed were antimony powder, granular bismuth metal, silicon powder, tellurium powder, 30-mesh granular tin, and 20-mesh granular zinc.

METALLIC OXIDES. Cerium dioxide was prepared from the oxalate and was probably contaminated by other rare earth oxides. When nitrogen dioxide mixtures, 2 to 50 per cent nitrogen dioxide, were passed through this preparation at 300° C., the nitrogen dioxide was completely removed from the gas stream. Unfortunately, use of a packing of cerium dioxide in place of lead dioxide in analyzing samples of benzoic acid gave consistently low results for carbon, although cerium dioxide has been used as an oxidation catalyst in macroanalysis (*12*).

A sample of special lead dioxide intended for use in microanalysis failed to remove all the nitrogen dioxide from a 2 per cent mixture passed through at the rate of 5 cc. per minute. This is further confirmation of Corwin's statement (*6*) that dry lead dioxide does not completely absorb nitrogen oxides. Powdered manganese dioxide removed nitrogen dioxide in 50 per cent mixtures but, since it is said (*6*) to be liable to the same sources of error as lead dioxide, it was not further investigated Thorium dioxide served to remove lower concentrations of nitrogen dioxide completely but failed at higher concentration. Analysis of benzoic acid using thorium dioxide gave low results for both carbon and hydrogen.

Samples of zirconium dioxide, prepared from zirconyl chloride and kept at a bright red heat, seemed to remove nitrogen dioxide completely. Analysis of 3,5-dinitrobenzoic acid, using zirconium dioxide at a red heat in place of lead dioxide, gave results for carbon which were high by an amount approximately equivalent to the nitrogen in the sample if it had been absorbed as nitrogen dioxide by the Ascarite. Analysis of the same compound using a permanganate–sulfuric acid solution between the Anhydrone and Ascarite tubes gave satisfactory results for carbon. Analysis of the permanganate–sulfuric acid solutions for nitrogen gave low results. The hydrogen results were satisfactory in all cases.

Summary

The defects in the use of lead dioxide in organic combustion analysis and suggested substitutes for lead dioxide are discussed.

It is believed that the method used for the determination of carbon and hydrogen in nitrogen-containing compounds has been improved by substituting for the lead dioxide in the combustion tube a sulfuric acid solution of potassium permanganate, or of some other oxidizing agent, which is placed between the absorption tubes used for water and carbon dioxide. Potassium permanganate and sulfuric acid are readily available in any laboratory and are considerably cheaper and more convenient to use than other proposed nitrogen oxide absorbents which are similarly placed in the absorption train, while apparently equally efficient. If a method can be found for converting all the nitrogen in the sample to nitrogen oxides, the proposed method will suffice for the simultaneous determination of carbon, hydrogen, and nitrogen. An added advantage of the proposed method is that it permits the use of a simplified combustion tube filling, whether or not compounds containing nitrogen are being analyzed.

In seeking a substitute for lead dioxide which could be used in the combustion tube or in the absorption train, various metals and metallic oxides have been tested for their ability to decompose or absorb nitrogen oxides. Although some of the metallic oxides satisfactorily remove oxides of nitrogen, their use is excluded since unsatisfactory carbon and hydrogen values are obtained when they are present.

Acknowledgment

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MODERN LABORATORIES

The American Tobacco Company Research Laboratory

CLAIBORNE E. BROGDEN, American Tobacco Co., Richmond, Va.

THE research facilities of The American Tobacco Company were greatly expanded with the completion of its new building at 400 Petersburg Turnpike, Richmond, Va. The building was designed by Francisco and Jacobus, of New York, and erected by the Wise Contracting Company of Richmond. The cost of the building and fixed equipment was approximately 95 cents per cubic foot.

The building, which consists of one story and full basement, measuring 135 by 110 feet, is of modified Georgian design, the exterior of buff brick with limestone trim. The exterior doors and sash are of wood painted white. The interior shows the influence of the Williamsburg restoration. The vestibule is paneled in teakwood, the lobby wainscoted to the ceiling and painted in old ivory. The library, which occupies 38 by 41 feet in the center of the building, consists of a conference room, from both sides of which open reading alcoves with bookstacks between. The room is finished in matched walnut panels. The executive and general offices are wainscoted in walnut or paneled in wood, painted ivory. The ceilings of the library and offices are acoustically treated. The floors of the vestibule and lobby are of marble, the library of teakwood with ebony inlay, the private offices carpeted.

LABORATORIES. The laboratories on the first floor are arranged in units, or suites, around the building. These units correspond to the divisions under which the research department is administered—tobacco, essential materials, cigaret paper, physical standards, tobacco smoke, factory service, and biological. The laboratories are 24 feet in depth and vary from 9 feet to 35 feet in width. Ceiling heights vary from 10 to 15.5 feet. The walls are of glazed terra-cotta to a height of 5 feet 9 inches with painted plaster above. Laboratory floors consist of $^3/_{16}$-inch air-pad rubber over concrete. Lighting is furnished by daylight fluorescent tubes operating on three-phase 208-volt circuits.

LABORATORY FURNITURE AND SERVICES. Laboratory furniture is of birch flush construction finished in "burley" brown, with the exception of the biological division, which is furnished with white enameled lead-coated steel. Table tops are 1.625-inch birch, finished in black satin acid-alkali resistant enamel, excepting under hoods. Table tops under hoods are of black serpentine stone 1.625 inches thick. Balance table tops are of 1.625-inch enameled asbestos composition, which provides added weight and stability.

Hardware and fittings are made of brass, satin chrome-plated, excepting under hoods, where special acid-resistant alloy is used. All are of severely plain design for ease of maintenance. Services in each laboratory include steam, hot and cold water, compressed air, gas, vacuum, 120- and 208-volt alternating current, and distilled water. All service lines are fully enclosed, so that only the fixtures are visible. Brass pipe is used throughout the building for hot and cold water. Ceramic drains for waste are used, except for lead connections from the floor level to laboratory sinks.

Sinks and drainboards of one-piece construction are of black vitrified ceramic ware, set in mastic at the joints with table tops to prevent moisture reaching unprotected surfaces. Acid-proof ceramic drip trays, connected to the acid drainage system, are provided beneath all sinks, so that traps and wall pipes may be serviced without damaging furniture or floors by contact with corrosive liquids. These trays also serve as an excellent storage for stock bottles of concentrated acids.

In addition to housing all mechanical equipment in connection with the building, the basement is provided with laboratories and other rooms for special equipment. Laboratory furniture in the basement is of lead-coated steel finished in a pearl gray enamel with impregnated asbestos tops.

One room, 32 by 35 feet, is equipped with semiworks digesters, beaters, sheet formers, and other equipment for fabricating cigaret paper from raw material. This room has a separate ventilating system for exhausting directly to the outside. A processing room, equipped with all services for use with small-scale factory machinery, occupies about 2000 square feet of floor space. A drying and milling room for tobacco and other samples has been provided, as well as a special room for pulverizing coal and related samples. The grinding mills are housed in hoods connected with rotoclones which remove 98 per cent of the dust, returning clean air to the rooms. One laboratory is equipped with a Kjeldahl wet-ashing unit provided with a Duriron vent directly to the roof. Moisture ovens having a capacity of over 500 samples a day occupy one room. A machine shop equipped with lathes, drill presses, and other necessary shop facilities occupies 24 by 30 feet.

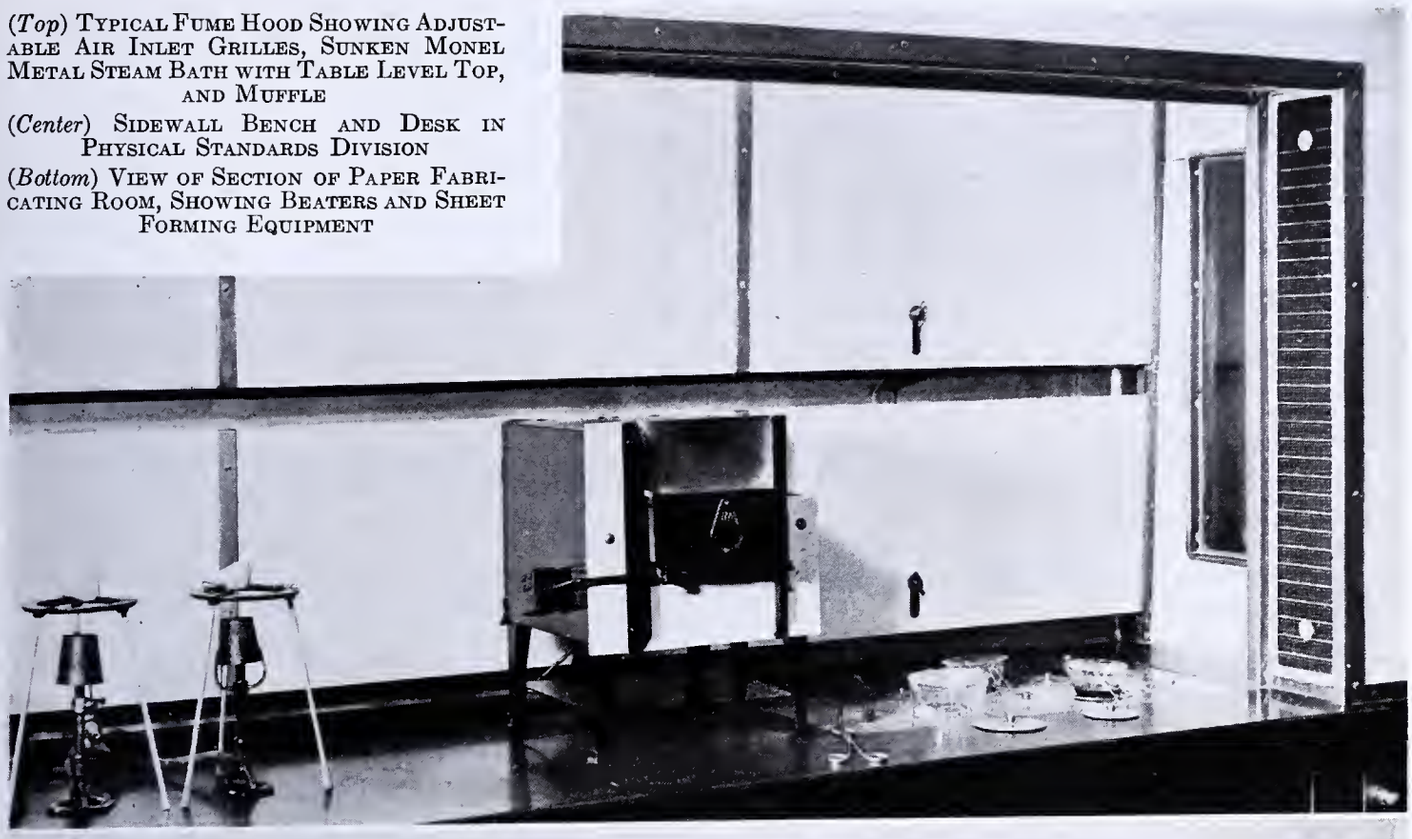
Stockrooms for glassware and chemicals, a storeroom for samples which are retained for reference, stationery stock room, record vault, shipping room, glassware washroom, sterilizing room, as well as locker rooms and showers for employees, are also located in the basement.

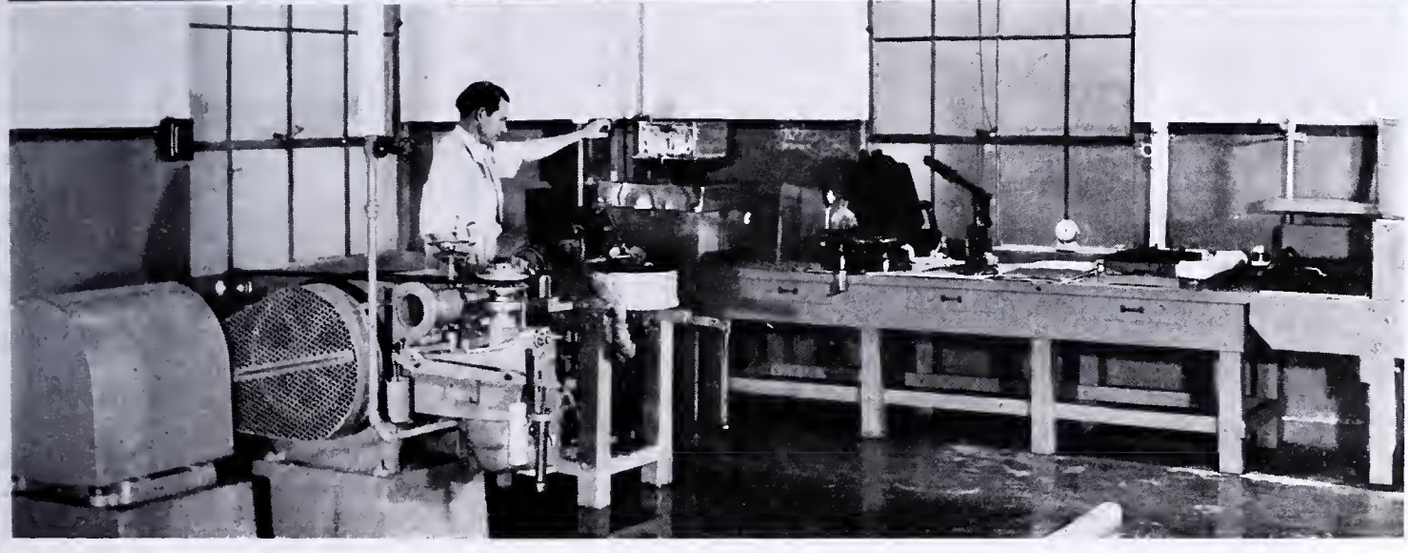
HEATING AND AIR CONDITIONING. The main floor is completely air-conditioned with controls in individual rooms or suites. The system is designed for both winter and summer conditioning—that is, dry bulb temperature and humidity will be automatically controlled throughout the year. All the air supplied to the laboratory through the air-conditioning system is thoroughly washed by means of a spray-type de-

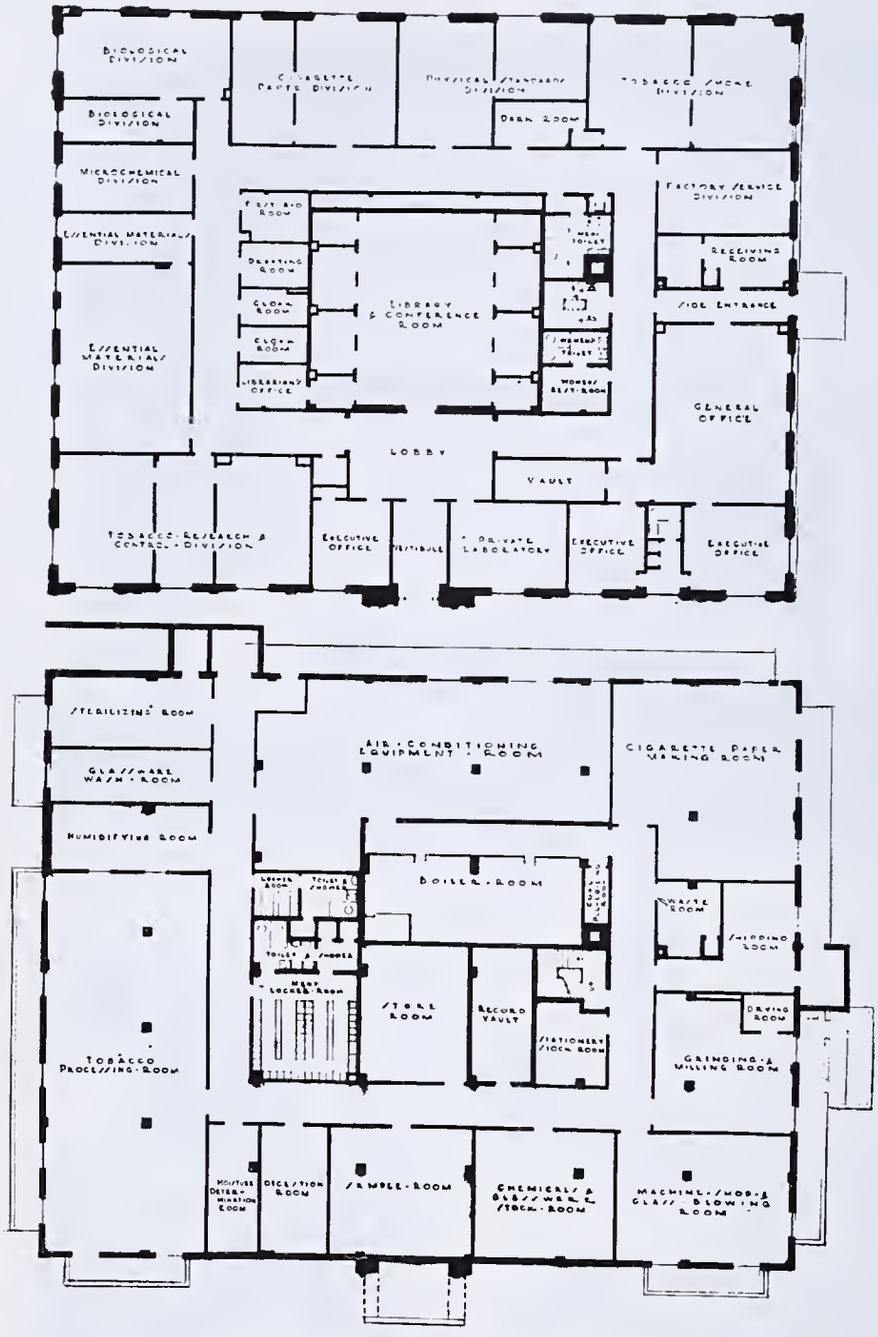
(*Top*) Typical Fume Hood Showing Adjustable Air Inlet Grilles, Sunken Monel Metal Steam Bath with Table Level Top, and Muffle

(*Center*) Sidewall Bench and Desk in Physical Standards Division

(*Bottom*) View of Section of Paper Fabricating Room, Showing Beaters and Sheet Forming Equipment







FLOOR PLAN OF MAIN FLOOR AND BASEMENT

humidifier, then filtered. The dehumidifier is fabricated of stainless steel, ensuring freedom from corrosion and minimum maintenance costs. The fan supplying conditioned air to the first floor has a capacity of 28,000 c. f. m., equivalent to a complete change of air every 6 minutes. Since the heat load is far greater in some rooms than in others, the quantity of air made available to each has been regulated accordingly. The system is divided into fourteen zones to provide for variation of internal heat load from laboratory equipment and of external load as the direct rays of the sun travel around the building.

Requirements vary from comfort conditions in the offices to very accurate control of both temperature and relative humidity—for example, measurement of the physical characteristics of cigarets and tobaccos requires the maintenance of relative humidity within ± 0.5 per cent and similar exactness in temperature control. Smoke research, while demanding less precise control, requires more flexibility, with facilities for changing relative humidity over a fairly wide range and maintaining it at any prescribed level during a series of experiments. Since the duties of each division are specialized, facilities have been provided which are peculiar to its needs. Individual rooms are equipped with special humidifying equipment for control at various levels. The conditioned air is introduced into the laboratories through adjustable openings in the window sills. The air, therefore, passes up across the window where the greatest heat change occurs. This avoids direct drafts on both worker and experiment.

Heat is supplied by two 36-horsepower low-pressure boilers arranged for oil burning. An additional 10-horsepower boiler of the Scotch Marine type generates steam at 100 pounds' pressure for laboratory use. In general this is reduced to 40 pounds' pressure and lower pressures for special uses.

Refrigeration is furnished by three compressors, each having a capacity of 40 tons of ice daily. They are supplemented by evaporative condensers which eliminate a cooling tower or large quantities of city water for condensing purposes.

A tobacco humidifying and aging room, insulated with 4-inch cork, has an individual air-conditioning system designed to control a dry-bulb temperature at any prescribed level from room temperature to 140° F. and to maintain any desired relative humidity up to 90 per cent.

An important and novel feature of the air-conditioning system is the development of air-conditioned fume hoods—that is, they are provided with a supply of air equivalent to about 90 per cent of the volume exhausted. Individual fans for both supply and exhaust for each hood are installed in the attic. The supply air enters through adjustable grilles around the top and sides of the opening and acts as an air screen against the escape of vapors. Normally, the hoods would need to be supplied with conditioned air from the laboratories, thus unbalancing the system. Since their requirement is approximately 35 per cent of the entire air-circulating capacity of the system, it would have been necessary to add a greater amount of refrigeration to accommodate this additional load. Their practicability has been fully demonstrated by use.

Exteriors of the hoods are finished in baked acid-alkali resisting enamel of a color to blend with the laboratory walls. The interiors of the hoods are lined with heat-resistant glass with Monel trim.

Titration assemblies are of special design, the frames being constructed of stainless steel. At the back of the assembly is an opalescent glass lighted by fluorescent tubes which provide permanent uniform lighting of correct intensity, well adapted to reading burets and end points.

All pegboards over wash sinks are finished in light-colored acid-resisting enamel to match the walls.

Distilled water tanks are concealed at the ends of center tables under the reagent shelves. They are arranged for easy removal for filling with only the cock exposed.

Waste receptacles of stainless steel are attached inside of swinging doors at recesses and tables, wholly concealed but easy of access.

Service fixtures are of one special design to ensure uniformity in appearance. All parts subject to wear are easily replaced with stock parts. Fixtures for air, vacuum, and steam are fitted with throttling nuts to ensure ease of control. Hand wheels are of black Bakelite of two-piece sanitary type with the proper initial to identify the service.

Fluorescent tube lighting was selected because of its high degree of efficiency, especially important in an air-conditioned building. The average intensity of illumination in the laboratories is 35 foot-candles.

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Instrumental Methods of Chemical Analysis

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The remarkable advances which have been made in the application of instruments to the problems confronting the chemist have been the topic of two previous communications in the ANALYTICAL EDITION (October, 1939 and 1940). In October, 1940, an initial treatment was concerned with a very general discussion of instruments of all kinds. It has become apparent that there is a real need for extensive, detailed discussions of various analytical methods. The editor has arranged for a series of expert reviews, several of which are in preparation. It seemed advisable to preface this series with some sort of preliminary survey or classification designed to illustrate the contemporary status of the field, to indicate the wide variety of techniques, and to dispel the notion, from which even the experts are not wholly immune, that progress is largely confined to a certain specialty or method. It is apparent that there is no "best" method for any kind of analysis.

The assignment of this problem to the author was based on no better recommendation than the last one—an interest in the subject and a pleasantly wide acquaintance with people who are doing important work in the field.

The collaboration which the author has had in this compilation is evident in the following pages. Several of the contributors of these examples are the experts whose detailed and authoritative expositions are to follow this review. Some serious defects and omissions are glaringly evident and the author's attempts to secure expert help and information were unsuccessful, in many cases for the reason satisfactory to every American—National Defense. This is no indication that those who could cooperate were not similarly committed and otherwise engaged in important and pressing work.

For the omissions and inaccuracies the author will plead the largeness of his design, with little hope for exoneration or clemency. The misplaced emphasis is intentional; in most cases the amount of discussion or detail is in inverse proportion to the state of development of the topic. Some instruments, which are fairly time-honored, are still treated in detail for reasons that are apparent. The literature dealing with some of the better known techniques is voluminous; in many it has become reduced to textbook practice. On those topics the few observations are opinionated and are not to be taken too seriously.

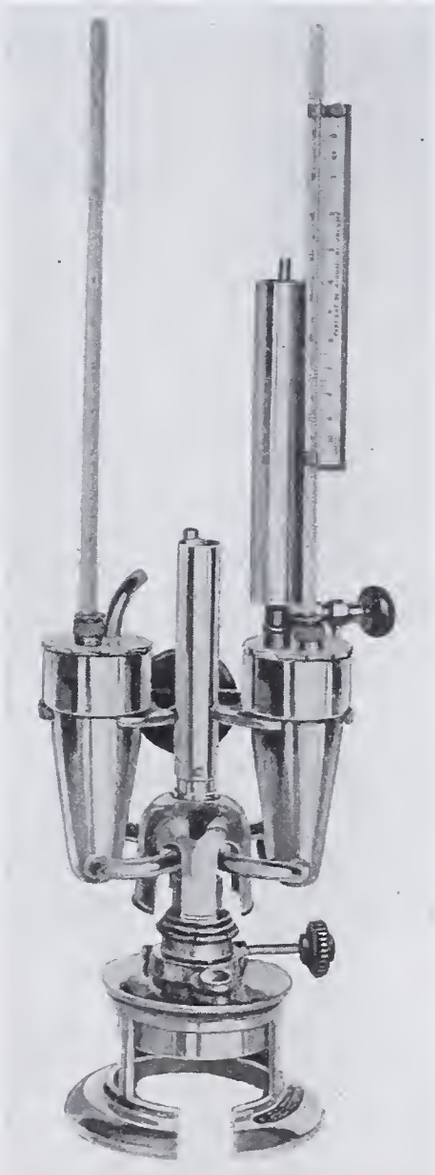
When a survey of modern analytical chemistry requires the description of instruments ranging from hydrometers to cyclotrons and it can be shown that these devices are all eminently practical and paying their way, one instinctively wonders how the modern analyst is to be appraised. It is evident from the contemporary scene that his former slogan "dry-ignite and weigh" no longer carries him through the day's work. He must become familiar with a bewildering array of techniques and at least moderately acquainted with the dialect of the physicist and engineer. By and large, he has done this and acquired a reputation for a broad outlook and wide range of experience none too common in other branches of chemistry.

IT WILL be necessary to explain the title of this review and the author's interpretation of its meaning. To be sure, a precedent exists (*165*, *259*) for the use of "Instrumental Methods. . . ." but a special intent and purpose lie behind its use. Avoiding quibbles about the strict meaning of "instrument" and its Biblical, Shakespearean, and musical uses, it is generally considered to imply a tool, aid, adjunct, or means of accomplishing some purpose. These are too general for the author's purpose and he has tried to restrict the term to the interpretation preferred by instrument engineers. Although it has not been possible to adhere rigidly to the adopted definition in all cases, the concept may be illustrated by a specific example.

It is well known that the boiling point of a binary liquid mixture can be related to its composition either on the basis of theory or, if pronounced deviations from ideality exist, on an empirical basis. If the boiling point of an unknown sample were measured and appropriate corrections were made for the barometric pressure, the composition could be calculated from the standard boiling point–composition curve. This is a physical method of analysis based on a definite property of the system. The device shown in Figure 1 has extended this fundamental idea to the point where we may designate it as an instrumental method of analysis. In this case two liquids are boiling at the same time, the unknown liquid and pure water. When a steady state has been reached in the boiling process, the zero of a sliding

scale on the thermometer which measures the boiling point of the unknown is set to the observed boiling point of the water thermometer. The position of the first thermometer is now read off from the adjacent scale which is calibrated not in degrees but in percentage composition (in the specific instance shown here per cent of alcohol by volume).

The improved technique has eliminated the need for barometric correction and the instrumental indication is in terms of the desired constituent. A physical method of analysis has been converted into an instrumental method of analysis.



Courtesy, C. J. Tagliabue Co.

FIGURE 1. EBULLIOMETER

Without intending to claim that we have hereby discovered a startlingly new principle, it does emphasize a point of view which has very important practical consequences. A rough analogy is to be found in the very common practice of calibrating volumetric solutions in terms of per cent of the desired constituent, even making due correction for a standard blank, instead of using the more formal scheme of normal or molar solutions. Although all devices are not conveniently designed to give a direct answer, it is to be regarded as the ultimate goal of a true instrument. In practice it boils down to the fact that in practical work computations must be reduced to a minimum, and although these may be the delight and pleasure of the research man the burden need not be tolerated if a comparable degree of ingenuity and skill can be exercised in the original design. In the following pages there are numerous examples, some of them time-honored, in which this ideal has been achieved; in others the need is so apparent that it at once explains the comparatively rare use made of the method despite its other attractive features.

If the intent of this term has been made clear it follows that many extremely important adjuncts to analysis cannot be considered here. That they are eminently worthy of discussion as important aids goes without saying. In addition, it will be found that we have not adhered consistently to our formulated definition of the instrumental method. Such methods are included nevertheless because by their nature they possess the inherent possibility of such development.

Density

Density is an unspecific property, but in systems of known composition it may be used as an accurate means of specifying concentrations (*57*). The simplest instrumental indication is afforded by hydrometers, and these devices are not only made to read specific gravity directly but may be obtained in a form which is calibrated directly to give the desired information. Typical examples are shown in Figure 2. Among some of the special scales we note alcohol, calibrated in per cent or in per cent of proof spirit; and scales to read calcium chloride, glue, salt, milk, sugar, sirup, or tanning liquor.

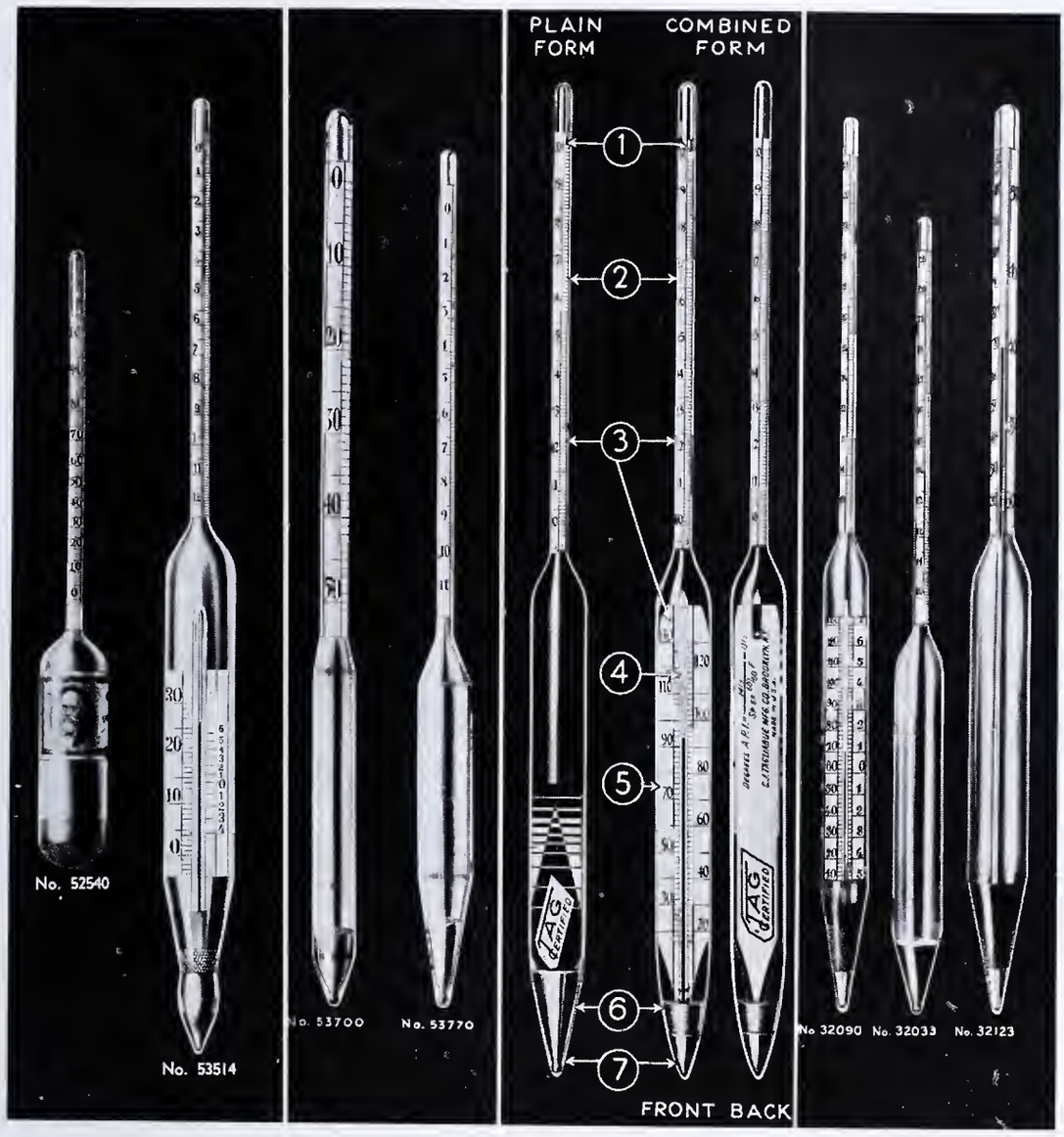
Precision hydrometers cover a restricted range and can be provided in a practical and technical form reading to one unit in the third place of specific gravity. This degree of precision translated into specific-purpose instruments might be illustrated by a precision sugar hydrometer covering a range of 69 to 81 per cent by weight which will give a reliable indication to 0.1 per cent. The proper use of hydrometers has been specified by the National Bureau of Standards and among other conditions assumes careful temperature control, equality of temperature of hydrometer and the liquid, and the use of the hydrometer with the liquid for which it is intended, because different surface tension effects may be revealed despite a common value of the density.

SPECIFIC GRAVITY BALANCE. The principle of the hydrometer may be extended with an increase of precision of about one order of magnitude by using a simple balance. Thus the Westphal balance (Figure 3) will read to about one unit in the fourth decimal place of specific gravity. This device becomes a means of a direct instrumental method of analysis if the scale is calibrated not in specific gravity units but in terms of the desired quantity.

Ultracentrifuge

A very long extrapolation from these simple devices is illustrated by the ultracentrifuge. This instrument is one of our most important means of studying substances of high molecular weight and is one of the few methods whereby information can be obtained from such complicated substances as proteins and polysaccharides. The specific sedimentation velocity is a constant characteristic of the dissolved unit, but it cannot be used without further information in calculating molecular weights on account of shape factors. Such information can be obtained, however, by a correlation between this quantity and diffusion constants.

INSTRUMENTS AND METHODS. Two notable examples of this equipment are illustrated from the laboratories of well-known investigators in this field: Figure 4, the ultracentrifuge of the Svedberg type (*270*), with rotor cell and bearing housing, and Figure 5, the ultracentrifuge laboratory from the camera end of the instrument. The process of centrifugal sedimentation is followed by allowing light to pass through



Courtesy, C. J. Tagliabue Co.

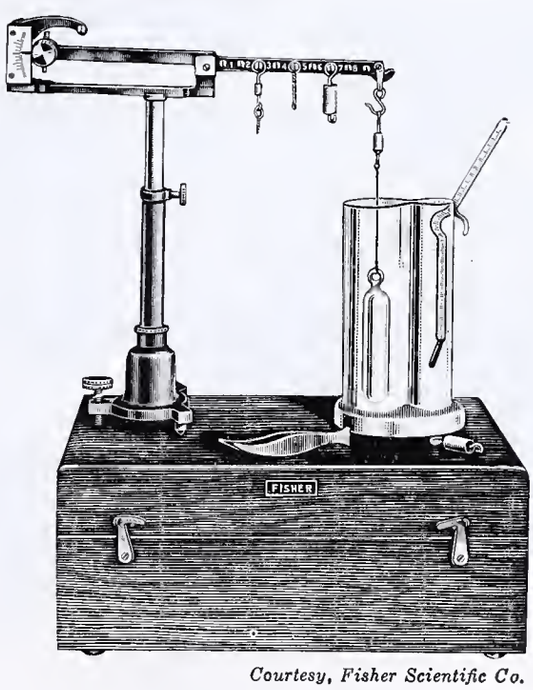
FIGURE 2. SPECIAL-PURPOSE HYDROMETERS

windows in the rotor and photographing the position of the boundary between solvent and solution at stated intervals. Either the light absorption or the scale method (Lamm) may be used. Figure 6 shows an analytical ultracentrifuge for the study of plant and animal viruses and of the protein components of sera. This instrument is of the air-driven type (*18, 306*).

APPLICATIONS. Density methods have been widely used and in principle are well suited to the automatic recording and telemetering of information of analytical interest. Dozens of methods and uses of this type are described by Gmelin (*67*) and in engineering texts. The ultracentrifuge in conjunction with related techniques such as absorption spectra and electrophoresis has afforded information on the nature of high-molecular-weight compounds where ordinary analytical methods can give either no answer or at least only approximate information. The discussion of density has been limited here to liquids and solutions. It is more completely discussed in connection with gas analysis.

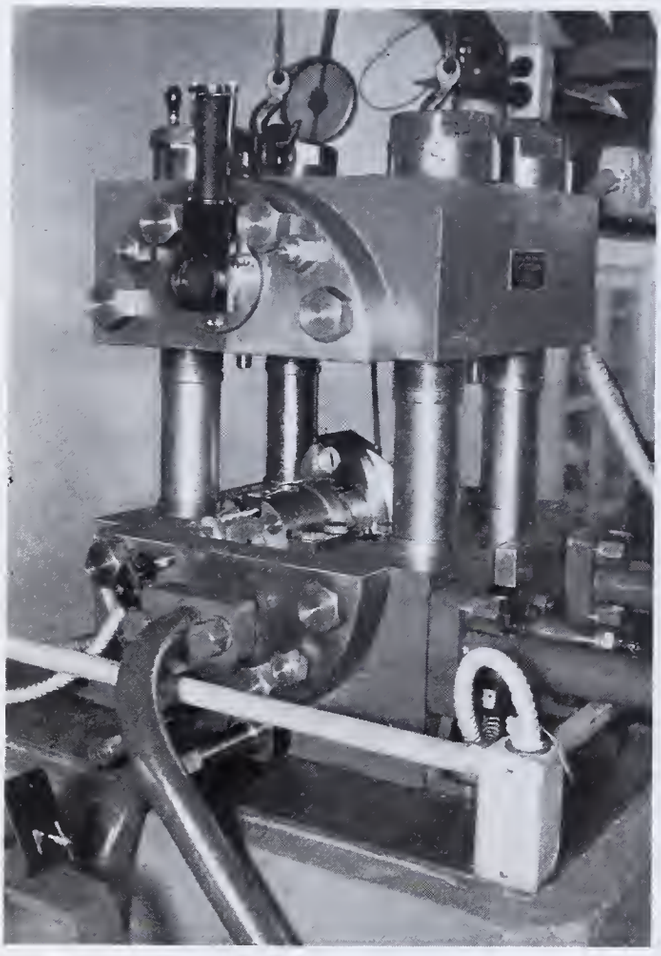
Thermal

Thermal methods include boiling point elevation and freezing point depression as well as the emission or absorption of heat accompanying chemical changes. These methods in general have limited analytical application but a few are



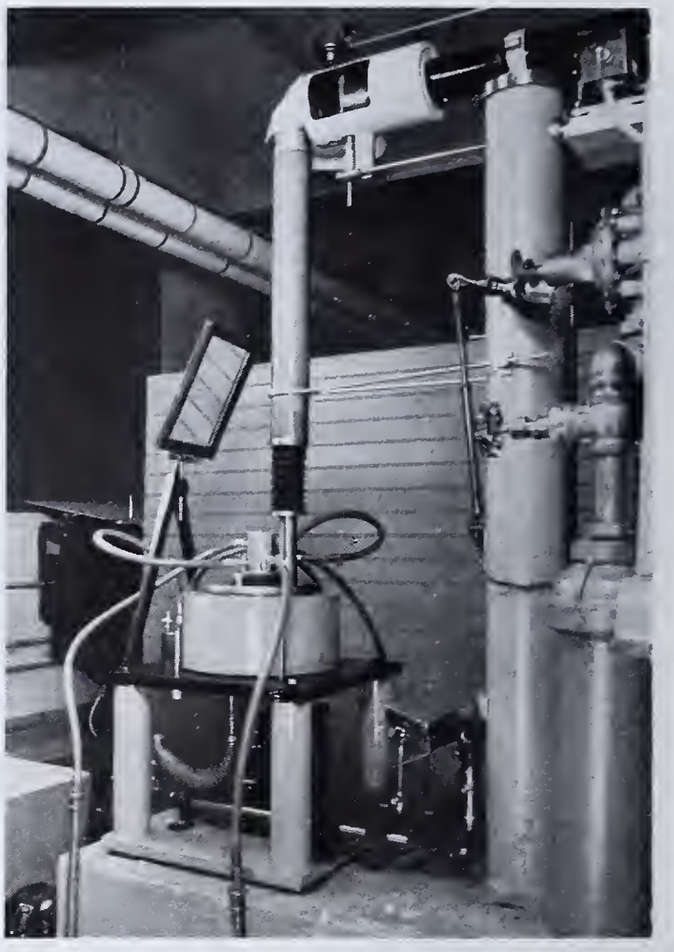
Courtesy, Fisher Scientific Co.

FIGURE 3. SPECIFIC GRAVITY BALANCE



Courtesy, J. W. Williams, University of Wisconsin

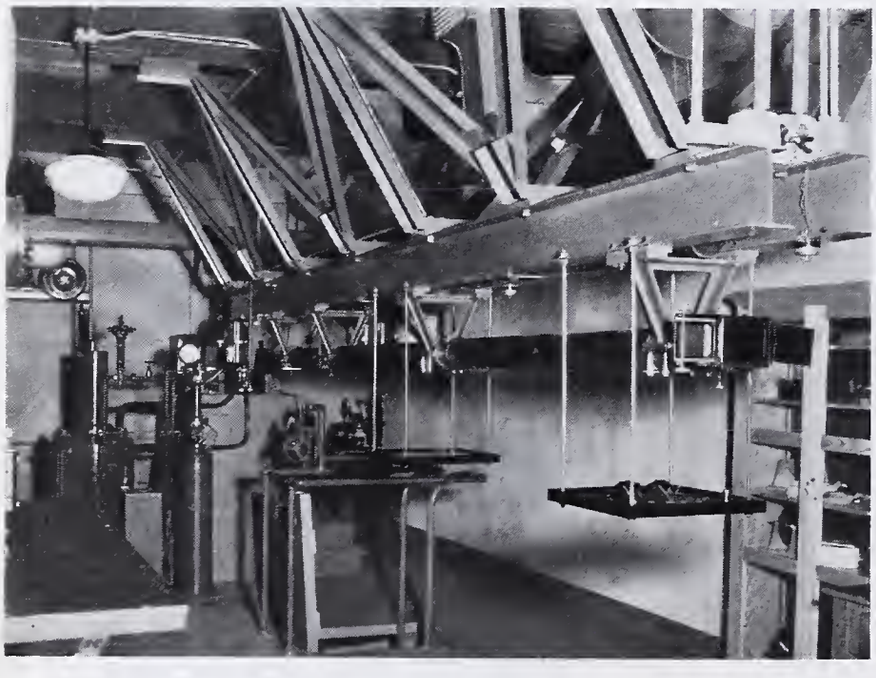
FIGURE 4. ULTRACENTRIFUGE, SHOWING CENTRIFUGE CASING, ROTOR, CELL, AND BEARING HOUSING



Courtesy, R. W. G. Wyckoff, Lederle Laboratories, Inc.

FIGURE 6. ANALYTICAL ULTRACENTRIFUGE

worthy of mention. The ebulliometer has been mentioned above. The freezing point depression for ideal solutions can be related simply to the composition, provided the molecular weight and the freezing point depression constant are known.



Courtesy, J. W. Williams, University of Wisconsin

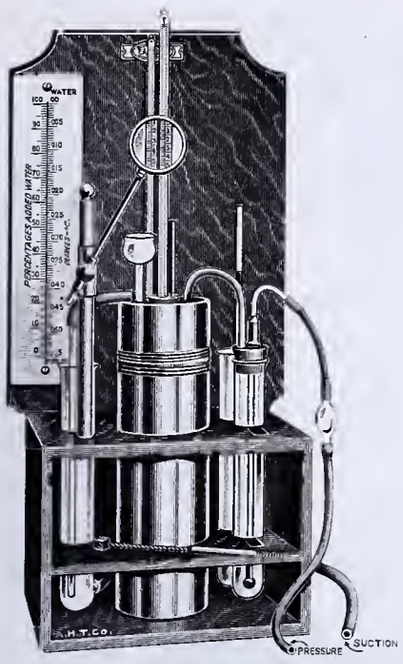
FIGURE 5. ULTRACENTRIFUGE LABORATORY, SHOWING CAMERA IN RIGHT FOREGROUND

The use of cooling curves in phase rule studies, particularly metallurgical, are well known and furnish information which is of importance in establishing the nature and composition of the various solid phases. The thermal techniques which are discussed under gas analysis might be included here.

INSTRUMENTS. Changes in temperature or the heat evolved in a process can be measured in a great variety of ways and are discussed in many standard works. We may mention in passing the use of gas and liquid thermometers, thermoelectric and resistance thermometry, and radiant heat by the thermopile and bolometer.

APPLICATIONS. A useful application of the freezing point method is illustrated in Figure 7, which shows the Hortvet cryoscope used for determining the amount of added water in milk. The freezing point is measured with a short-range thermometer graduated in 0.01° divisions. The freezing is accomplished by the evaporation of ether by a current of air, for which purpose a filter pump may be used. A scale attached to the instrument converts the observed temperatures directly to percentages of added water. The use of this instrument has been described in several places.

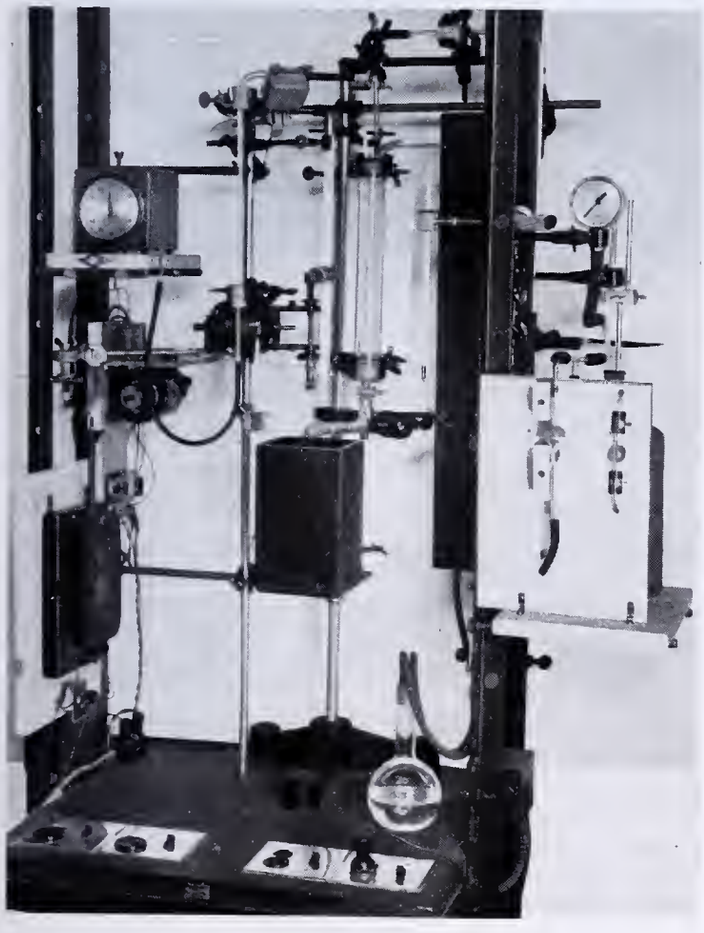
A measurement of the amount of heat evolved during the progress of a titration has been used on a number of occasions.



Courtesy, Arthur H. Thomas Co.

FIGURE 7. CRYOSCOPE

The first practical use seems to have been made by Bell and Cowell (*16*). Other examples have been described by Somiya (*264*). In general, the titration is carried out to within a few per cent of the end point and the change in temperature after small additions of reagent is noted. The measurements are continued somewhat beyond the end point and its true location is determined graphically. Most of the cases considered by Somiya were concerned with concentrated or fuming acids and the temperature rises were considerable. He points out, however, that the water content of concentrated sulfuric acid can be determined more accurately by thermometric titration than by acidimetry. With more refined measurements this technique can be extended profitably to much more dilute solutions.

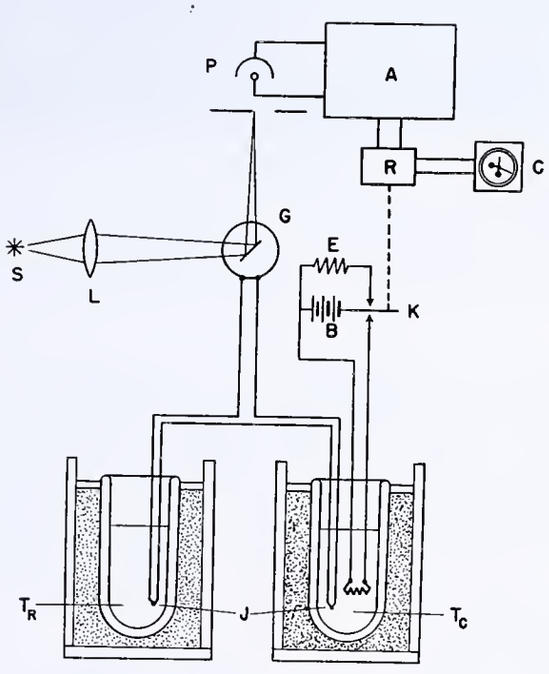


Courtesy, New York University

FIGURE 8. THERMOMETRIC TITRATION APPARATUS

Figure 8 shows an arrangement which has been used in the writer's laboratories for this purpose, with means for showing the titration both with the Beckman thermometer and by a differential thermoelectric method which is represented schematically in Figure 9. In the latter the heat evolution in the titration vessel is exactly and automatically compensated by the electrical addition of heat in the reference vessel. The multijunction thermocouple and galvanometer control this process through the agency of a simple photoelectric relay which switches a constant heating current on or off. A Telechron clock indicates the time during which constant heat is imparted to the reference cell. The elapsed time is therefore proportional to the heat which has been evolved as a result of the titration increment (Figure 10).

These methods are not particularly useful unless considerable heat is evolved or unless automatic means such as the one described are used to measure the temperature rises. Unless the results are automatically recorded and evaluated, the need for graphic computation of the end point has the same time-consuming drawback characteristic of other methods such as conductance titrations.



Courtesy, New York University

FIGURE 9. THERMOMETRIC TITRATION APPARATUS

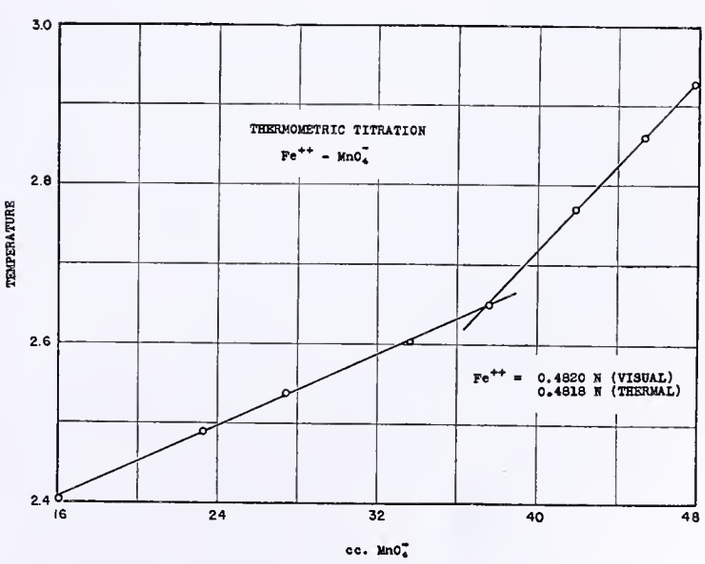


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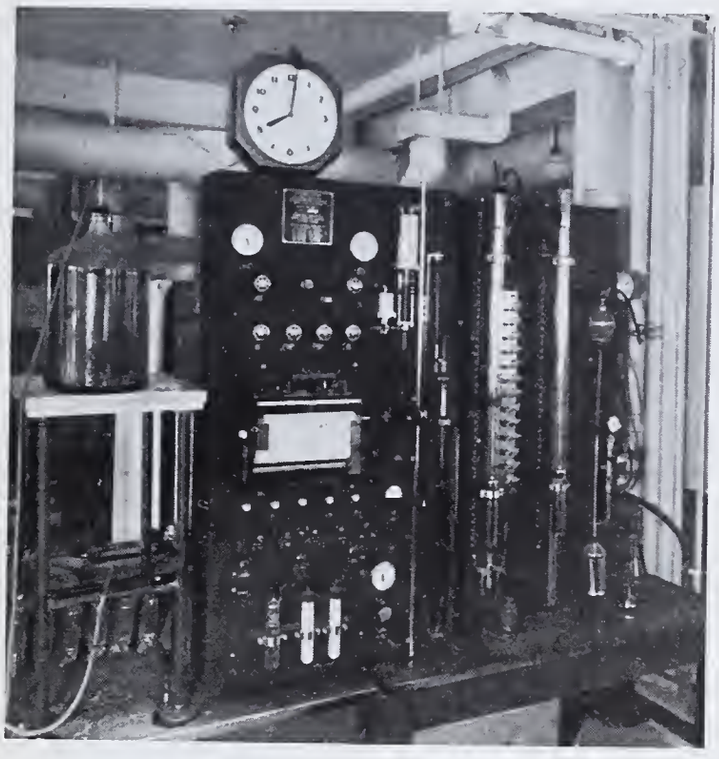
FIGURE 10. THERMOMETRIC TITRATION CURVE



Courtesy, Podbielniak Centrifugal Super-Contactor Co.

FIGURE 11. AUTOMATIC RECORDING LOW-TEMPERATURE FRACTIONAL DISTILLATION ANALYSIS APPARATUS

Installation in laboratories of Catalytic Development Co., Marcus Hook, Penna., for research and development on catalytic cracking

Distillation methods might be classified under this heading and throughout the history of chemistry distillation has been used as an adjunct in chemical analysis. However, completely automatic distillation techniques primarily for analytical purposes have been developed, a striking example of which is shown in Figures 11 and 12. This instrument provides a complete record of the distillation history from liquid air temperatures to 250° C. A schematic diagram of the distillation apparatus is shown in Figure 13.

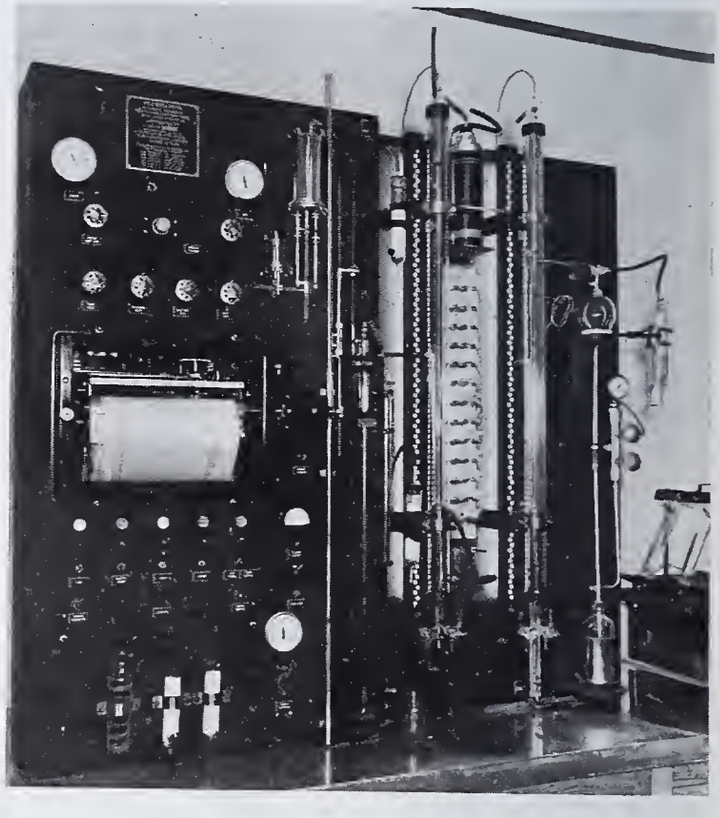
Analytical Molecular Still. The cyclic molecular still is the outcome of efforts to make the short-path high-vacuum surface evaporator into a tool for exact analysis and research. The apparatus in its present stage of development is shown in Figure 13A.

A is the distillation column contained within the glass condenser, *B*. The column is filled with oil which is stirred by the small air motor, *C*, while it is heated electrically through current leads shown on the right-hand frame support. The pressure in the apparatus is determined by the Pirani gage tubes, *D*, and meter, D^1. The substance to be distilled is placed in the reservoir, *E*, and circulated by the magnetic pump, *F*, and actuating box, F^1, through the preheated tube, *G*, which should be wound with fine Nichrome wire and fed by current controlled through a resistance. During circulation the distilland from *E* passes back through the water-cooled tube, *H*, into the upper reservoir, E^1. An iron ball separates the reservoirs and the ball is withdrawn when needed, by hand magnet (not shown). The vacuum equipment consists of high-speed condensation pump, *K*, connected to an intermediate booster, *L*, the reservoir, *M*, a freezing trap, *N*, useful in collecting solvents, and a mechanical pump not shown but generally situated below table at *O*. The high-vacuum system is connected to the still through the manifold box, *P*, and the wide return in tube *Q*.

To operate the still a liquid is placed in the reservoirs and the ball separating them removed to the side pocket, *R*. The liquid is circulated under gentle heat while the vacuum pumps are started. When the pressure has fallen to less than 10μ the temperature is fixed at a predetermined level and samples of the fraction are collected in the receiver, *S*. After one complete cycle (the ball having been placed in position at the beginning of the cycle) the stopcock, *T*, is manipulated, receiver *S* changed to a new one, stopcock *T* again manipulated, and the material in E^1 let down to *E* and circulated at a new temperature generally exactly 5° or 10° higher than the previous one. This series of operations is kept up until sufficient material has been collected, generally over a range of 120° to 200° C. The concentration of all constituents of interest is then determined in each fraction and the concentration multiplied by the weight of the fraction, which equals total yield, is plotted graphically against temperature. There results an elimination curve or series of curves, such as shown in Figure 13B.

Gas Analysis

The analysis of gas can be accomplished by a great variety of techniques, starting with the simple absorption methods, bell chamber equipment, electrolytic conductance, thermal conductance, and combinations of these methods preceded by preliminary chemical treatment, by the heat evolved in catalytic combination, or by optical methods, including absorption in the visible, ultraviolet, and infrared, as well as refractometric methods utilizing the interferometer. They range from the analysis of a fraction of a cubic millimeter to the completely automatic recording of gas composition on an industrial scale (*54*).



Courtesy, Podbielniak Centrifugal Super-Contactor Co.

FIGURE 12. AUTOMATIC RECORDING LOW-TEMPERATURE FRACTIONAL DISTILLATION ANALYSIS APPARATUS

Installation in laboratories of Lion Oil Refining Co., El Dorado, Ark., for analysis of natural gas and gasolines and for separate determination of paraffin and olefin in C_3 and C_4 cracked refinery gas fractions

THEORY. Our knowledge of the gaseous state is in a high state of development and the contributions from thermodynamics and the kinetic theory have provided very accurate equations of state. Several of the methods used for the analysis of gas are based upon straightforward applications of the theory. Others, such as thermal conductance and the heat of catalytic conversion, for the most part require empirical calibration; for, despite extensive theoretical investigations, the predictions of the properties of mixtures, especially in the case of thermal conductance, are not accurate

enough for analytical purposes. The wide range of applicability and the extreme sensitivity of some of the instrumental methods make this one of the most important branches of analytical chemistry.

Instruments and Methods. *Determination of Gas Density.* The measurement of gas density is extremely important and has formed the basis for many automatically recording systems. The gas density balance illustrated in Figure 14 is based on a measurement of the pressure of the gas necessary to bring a balanced float to a standard equilibrium position under conditions of constant temperature. Its uses have been described by Edwards (*62*). It is applicable to a wide range of systems, but since density is an unspecific property, in some cases the measurement must be preceded by chemical treatment. Numerous recording methods for engineering applications have been described (*71*). In one method the streaming gas properly throttled flows into one tube and displaces a suitable manometric liquid into another limb of the tube (*278*).

Among the dynamic methods the effusion velocity principle has been applied in a number of ways by allowing the gas to diffuse out through a pinhole orifice (*65*). The density and composition of simple mixtures can be inferred from the rate of effusion. Here, too, theoretical predictions are not too helpful because the mixture laws apply only with systems containing molecules with the same number of atoms and viscosity coefficients that do not differ too widely. As a rule empirical calibration is necessary. An example of this type of instrument is shown in Figure 15.

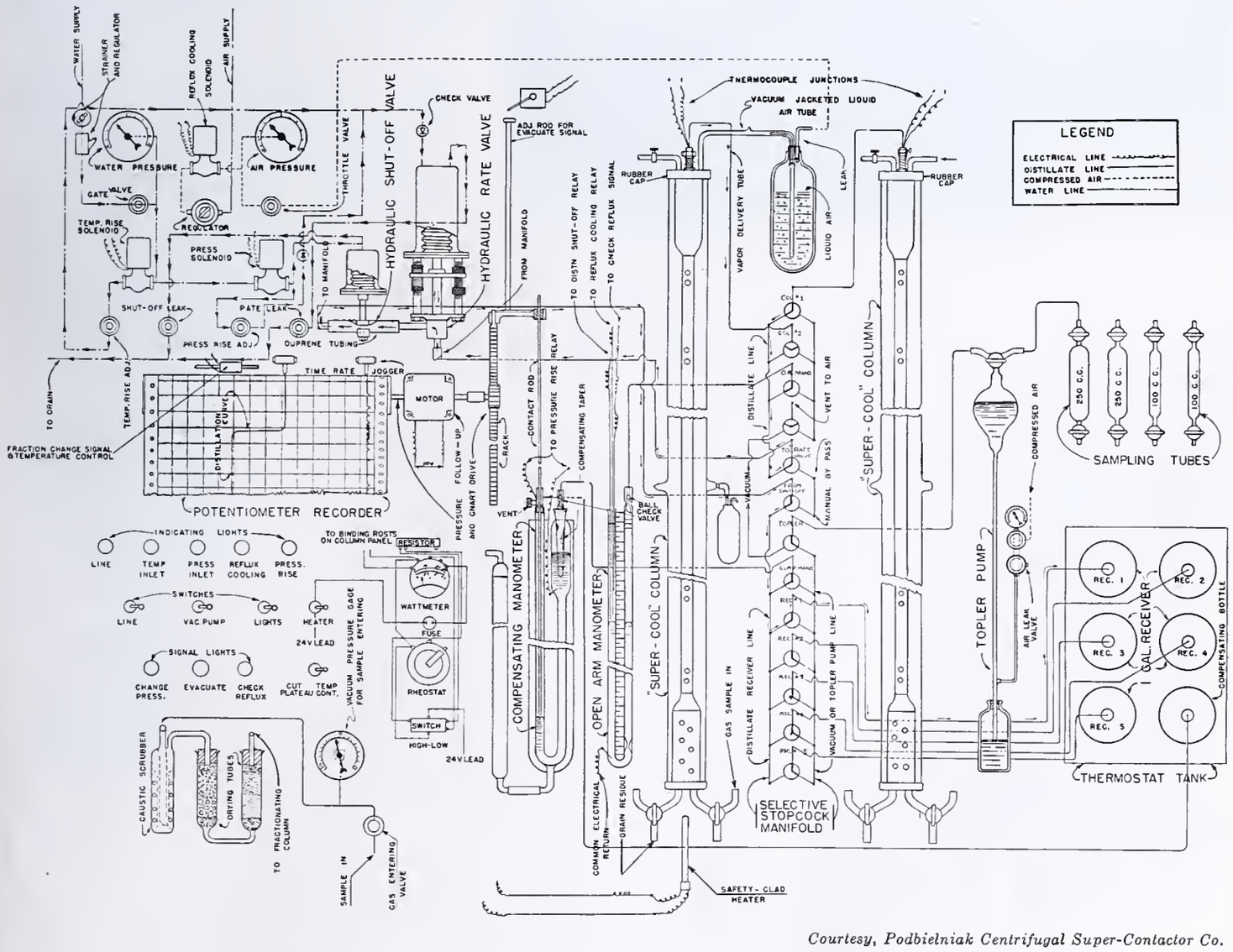
Effusiometers of the recording type employ a diving bell principle or floating plunger to actuate the recording pen. These arrangements are well suited for telemetering.

The Ranarex principle depends upon an aerodynamic coupling between a motor-driven ventilator fan and a similar fan which is connected with the indicating mechanism. The degree of coupling is a function of the gas density (Figure 16).

The many extensions and elaborations of these principles have been described (*57, 65, 71, 260,* and in standard engineering works).

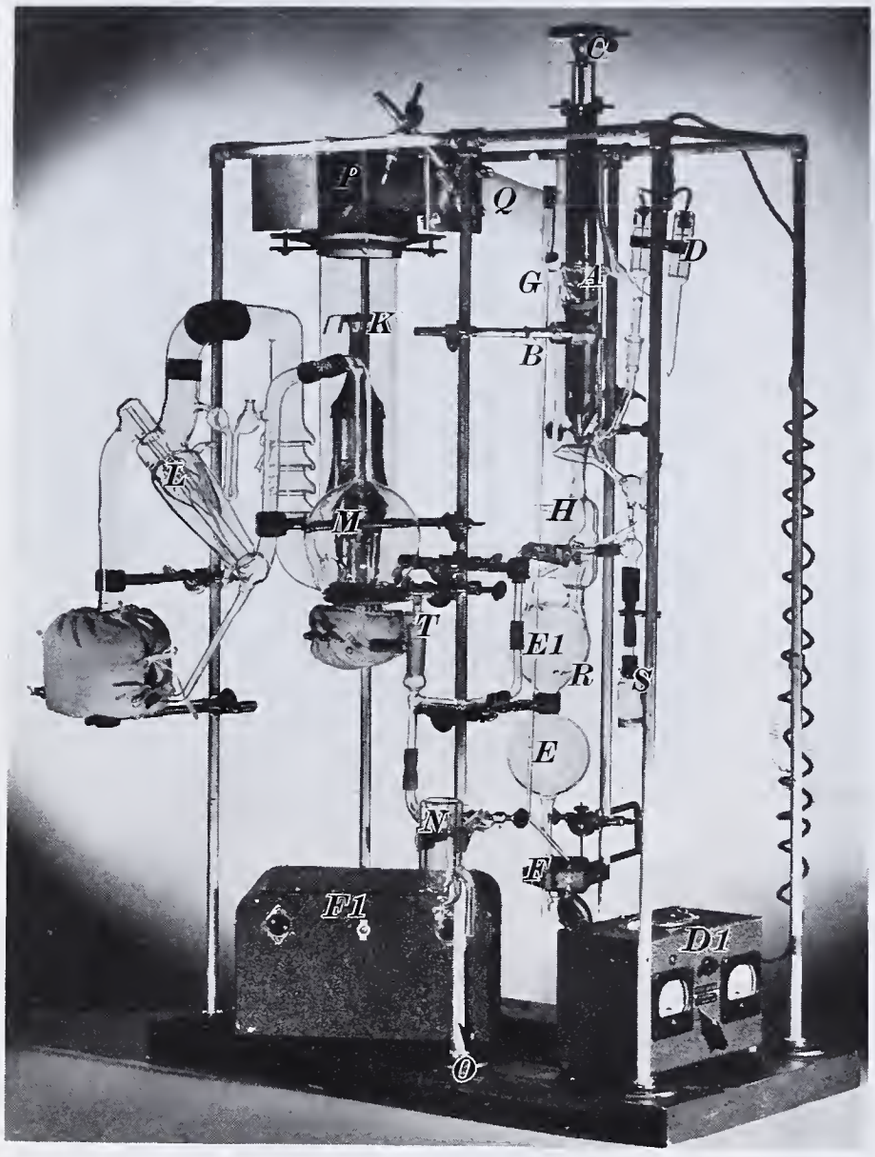
Absorption Techniques. The classical absorption techniques form the subject matter of many treatises and manuals (*65, 69, 92, 93, 115, 181, 212, 297, 301*). A typical example of a modern coordinated unit for the analysis of gases is shown in Figure 17. Very frequently a problem reduces to detection of a gas and a rough quantitative indication, particularly in the case of highly toxic or otherwise hazardous gases.

Figure 18 indicates a convenient portable hydrogen sulfide detector. The simple bulb aspirator draws a sample of the suspected air through a renewable detector tube. The reaction of hydrogen sulfide on the filling of the tube produces a color change, the extent of which is compared with an adjacent scale reading directly in per cent of hydrogen sulfide. The instrument indicates the actual concentration of the gas in amounts ranging from 0.0025 to 0.04 per cent by volume. The calibration is



Courtesy, Podbielniak Centrifugal Super-Contactor Co.

Figure 13. Schematic Diagram of Distillation Apparatus



Courtesy, Distillation Products, Inc.

FIGURE 13A. MOLECULAR STILL

based on the assumption that the bulb has been squeezed ten times, expanding completely after each aspiration. Figure 19 illustrates the convenient portable nature of the equipment for field work and the simple renewal with reagent cartridges.

One of the nicest examples of refinements in the general technique is to be found in the modern means for analyzing extremely small amounts of gas. Of these the systematic developments and studies by Blacet and Leighton (*19–23*) form an outstanding example. The apparatus which they have developed for this purpose is shown in Figures 20, 21, and 22. The merit of their equipment lies not merely in the small samples which can be accommodated but more particularly in the high degree of selectivity and precision. This is best illustrated by an excerpt from one of their papers (*22*) as shown in Table I.

TABLE I. ANALYSES FOR ACETYLENE IN THE PRESENCE OF PROPYLENE AND CARBON MONOXIDE

Determination	Volume of Sample	Theoretical	Acetylene Determined	Difference
	Cu. mm.	%	%	%
1	45.44	33.10	33.09	−0.01
2	40.75	33.10	32.98	−0.12
3	37.66	33.10	33.15	+0.05
4	40.82	33.10	33.11	+0.01
5	42.61	33.10	32.80	−0.30
			Av. 33.03	0.10

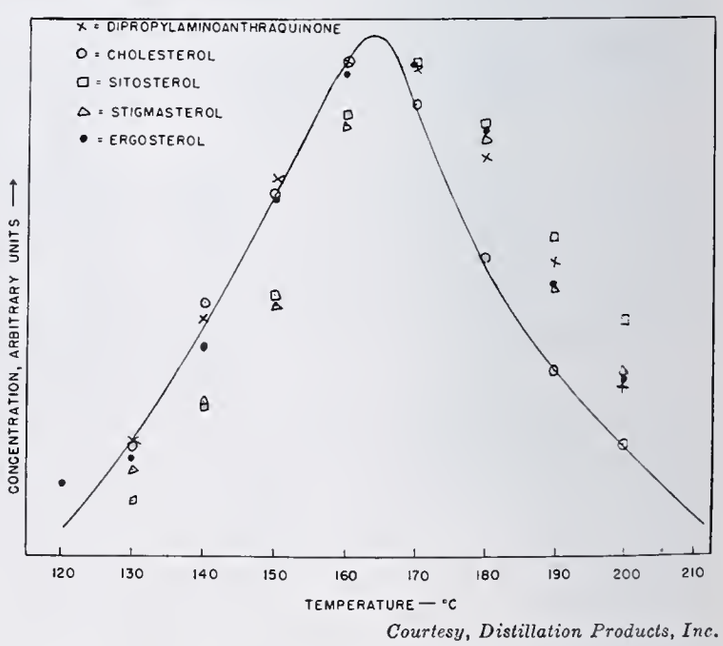
Another approach to the microanalysis of gases is to be found in the early work of Langmuir in analyses conducted at very low pressures. These are discussed in great detail in several monographs on high-vacuum technique (*60, 97, 206*).

Another approach to the microanalysis of gases is to be found in the Barcroft-Warburg manometer, which has widespread use in biological investigations. This equipment, examples of which are shown in Figures 23 and 24, is useful not only in measuring small amounts of gas but in following the kinetics of gas reactions. Some applications of the equipment, illustrated in Figure 23, have been described (*9, 146*).

Bell Chamber Methods. The general principle involved in this technique consists in trapping the gas under a floating bell in which the supporting liquid contains a reagent that absorbs the gas. Thus in the specific instance of a recording carbon dioxide meter the liquid is a concentrated solution of sodium hydroxide. As the carbon dioxide in the gas is absorbed the diving bell sinks and moves the indicating or recording element in proportion to its motion. This instrument is exemplified by numerous combustion recorders and in the medical field by metabolism apparatus (Figure 25).

This class of instrument is also suited to telemetering, in which case the moving element upsets the balance of an inductive bridge, or through related transfer mechanisms (*71*).

Electrical Conductivity. This technique is more properly discussed under electrolytic conductance, but instruments of this class are based on the absorption of the gas in question by a suitable electrolyte, after which changes in the conductance of the solution are measured. It has been used specifically for the measurement of water vapor, carbon dioxide



Courtesy, Distillation Products, Inc.

FIGURE 13B. ELIMINATION CURVE FOR MOLECULAR STILL

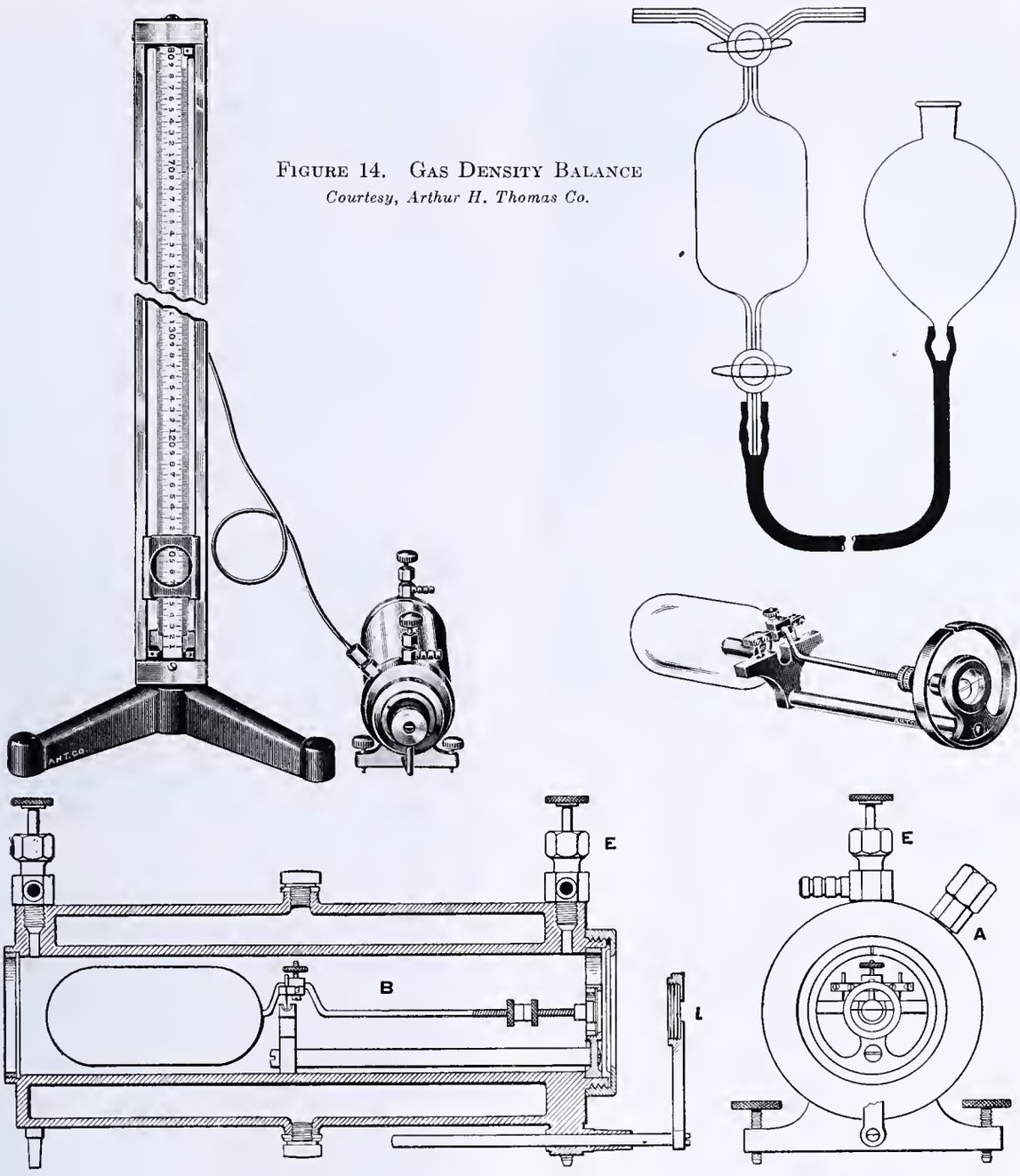


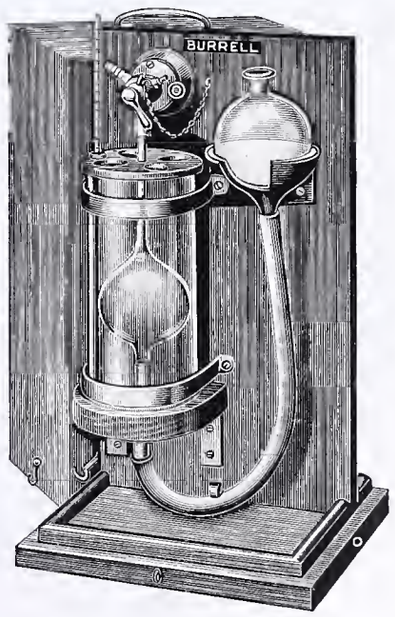
FIGURE 14. GAS DENSITY BALANCE
Courtesy, Arthur H. Thomas Co.

(*291, 300*), and sulfur dioxide, although in principle any gas which can be made to react with a suitable electrolyte may be used—i. e., carbon monoxide has been analyzed by this method by preliminary catalytic conversion to carbon dioxide, which is measured in the ordinary way. Equipment of this sort has been used to measure carbon dioxide in the conventional combustion train. Instrumental details are described in most treatises on conductometric analysis.

Thermal Conductance. This very important method of gas analysis is based on the thermal conductivity of the gas as a function of its composition. At atmospheric pressure the thermal conductance is relatively independent of the pressure and solely a function of the composition of the gas. At low pressures (10^{-2} to 10^{-5}) the thermal conductivity of a gas in a given cell is extremely sensitive to pressure and indeed forms the basis of a very sensitive pressure gage (Pirani gage).

The simplest thermal conductance gage consists of two contiguous chambers. Along the axis of each chamber is stretched a fine wire having a high-temperature coefficient of resistance, usually platinum. The wires form two arms of a Wheatstone bridge. One wire is surrounded by a standard reference gas and the other by the gas to be analyzed. The two wires are heated by the bridge current to a temperature of the order of 100° C. above the surrounding and the heat loss by gas conduction determines the effect of temperature of the wire, which in turn determines its electrical resistance and therefore the state of balance of the bridge.

The complete theory and practice of this principle have been described in many places and are the subject of an excellent monograph (*51*), which gives complete details of the underlying theory and the characteristics and particular advantages of the various bridge circuits.



Courtesy, Burrell Technical Supply Co.

FIGURE 15. GAS DENSITY BALANCE

Courtesy, The Permutit Co.

FIGURE 16. PORTABLE GAS DENSITY INDICATOR (*left*) AND INDICATING-RECORDING GAS DENSITY ANALYZER (*right*)

The suitability of the method and the degree of precision which is obtainable are governed primarily by the value of the specific conductance of various gases. Table III (*197*) of last year's review lists values for some of the commoner gases referred to air as unity. It has been pointed out very clearly by Daynes that these values and practically all those that have been recorded in the literature are to some extent dependent upon the apparatus and experimental conditions which were used for their determination. This is just another way of indicating that most instruments used for this kind of measurement do not conform strictly to the conditions implied in the definition of thermal conductance. It also illustrates why empirical calibration is necessary.

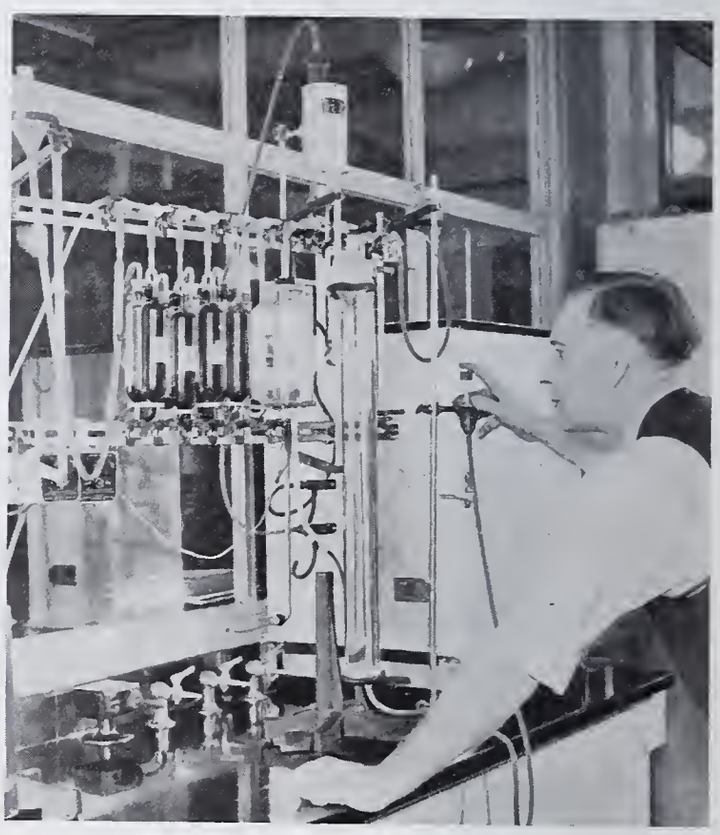
The many applications of this principle include equipment for the automatic analysis of dissolved oxygen in feed water (Figures 26 and 27), of carbon dioxide (Figures 28 to 31), and of sulfur dioxide (Figure 32). Perhaps one of the best indications of the wide range of utility is shown by the comparative records of Figure 33. Of particular interest is the trend toward the development of all-glass cells (Figure 34) which permit the use of relatively corrosive samples (*106, 226, 293*).

Heat of Catalytic Conversion. An extension of thermal methods which at least in some instrumental aspects is similar to the thermal conductance method is the utilization of the heat of reaction evolved when the gas in question is subjected to some chemical reaction (*81*).

Figure 35 (upper left and lower right) illustrates an instrument for the detection of explosive gas mixtures. This completely portable device uses a single filament on which the gas is catalytically oxidized or burned. The compensating resistor consists of a small lamp bulb. The process of combustion raises the temperature of the measuring filament and the electrical unbalance caused by the increase in resistance is indicated directly on the meter in per cent of explosive mixture. In this model a small piston pump is used instead of an aspirator bulb. Standard flashlight batteries are employed for heating the filaments.

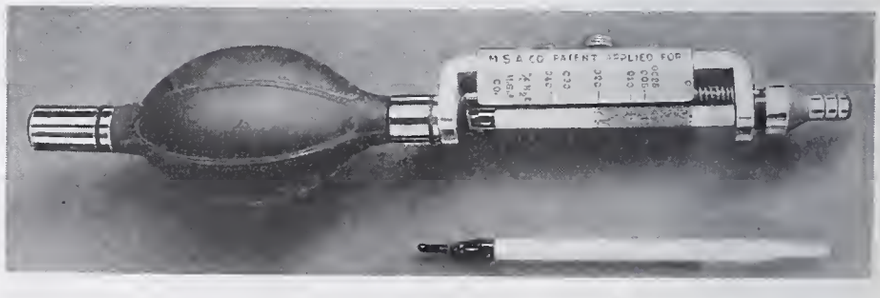
In the combustible gas indicator shown in Figure 35 (center and lower left) two platinum filaments are employed and the combustible gas is oxidized or burned on the surface of one of the filaments. The electrical unbalance is indicated on a scale calibrated to give readings directly in per cent of lower explosion limit concentration. A single indicator can be calibrated to give direct readings from one or several combustible gases and vapors, including petroleum, alcohols, ethers, acetone, natural gas, blast furnace or coke-oven gases, etc.

Another class of indicator is illustrated in Figure 35 (upper right). The carbon monoxide-bearing air is drawn into the



Courtesy, Burrell Technical Supply Co. and Buick Metallurgical Dept., General Motors Corp.

FIGURE 17. USE OF GAS ANALYSIS APPARATUS IN METALLURGICAL LABORATORY



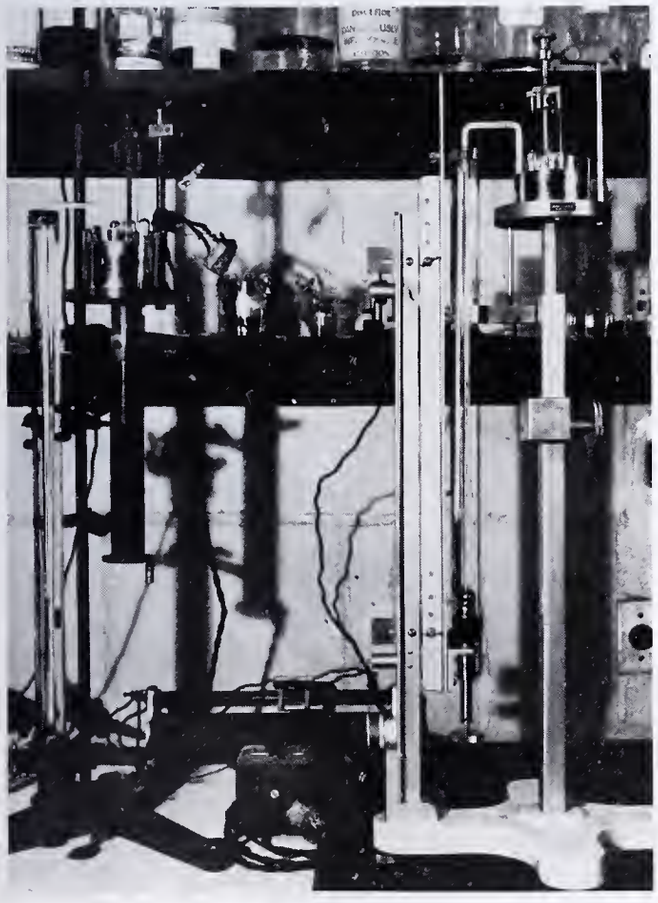
Courtesy, Mine Safety Appliances Co.

FIGURE 18. HYDROGEN SULFIDE DETECTOR

A hand-operated instrument for quick and accurate detection and measurement of low but dangerous concentrations of hydrogen sulfide.

Courtesy, Mine Safety Appliances Co.

FIGURE 19. HYDROGEN SULFIDE DETECTOR

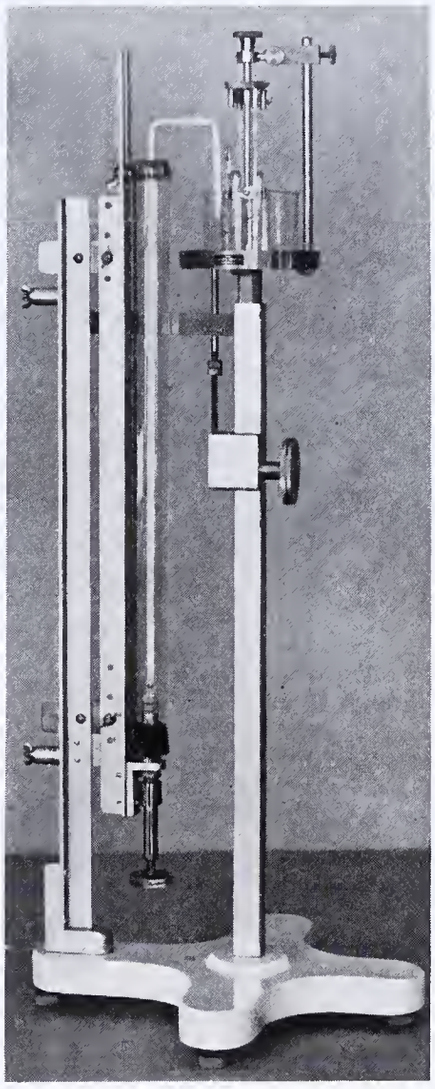


Courtesy, F. E. Blacet, University of California, Los Angeles

FIGURE 20. TWO FORMS OF BLACET-LEIGHTON APPARATUS FOR MICROANALYSIS OF GASES

(*Left*) Eimer & Amend style
(*Right*) Arthur H. Thomas Co. style

instrument by a motor-driven pump provided with a flowmeter indicator. The gas is drawn through a catalytic conversion chamber filled with Hopcalite. The heat of conversion to carbon dioxide is measured by the series of thermocouples in series with the indicating meter which is calibrated to read directly in per cent of carbon monoxide. The scale ranges from 0 to 0.15 per cent and can be read directly to 0.005 per cent and estimated to

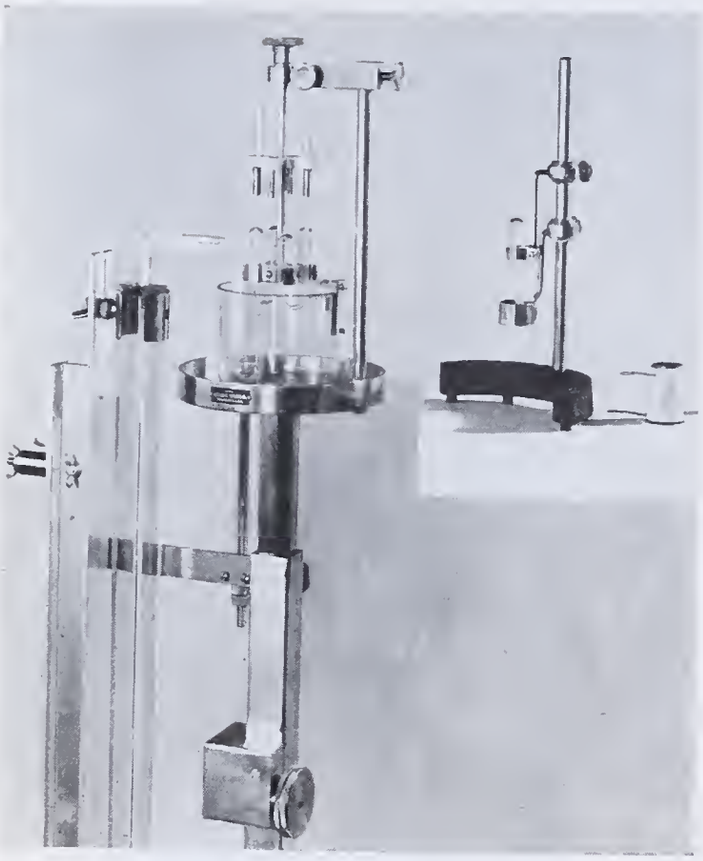


Courtesy, Arthur H. Thomas Co.

FIGURE 21. BLACET-LEIGHTON GAS ANALYSIS APPARATUS

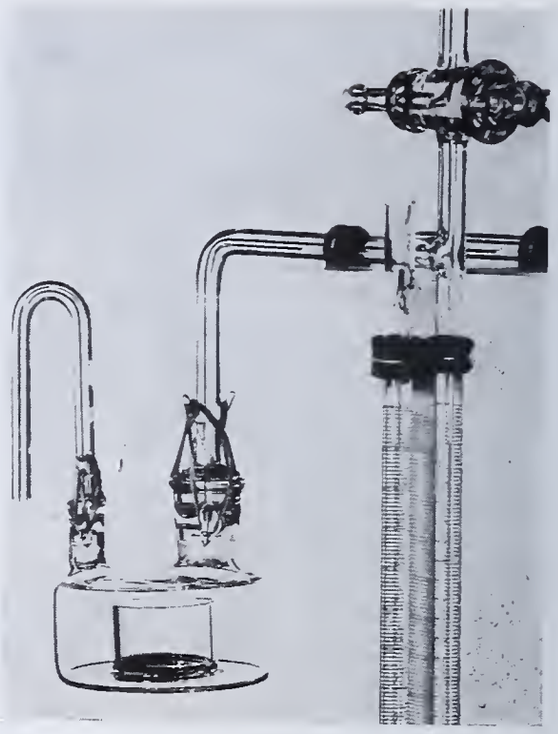
0.001 per cent. In a hand-driven model a small highly efficient four-cylinder radial pump develops proper suction for sampling and an automatic regulator maintains the correct sample flow which is indicated on a diaphragm-type pressure gage. The motor-driven type could be operated from any 6-volt alternating or direct current supply or from self-contained Edison storage batteries.

Two extensions of this principle, shown in Figures 36 and 37, provide automatic alarm features. The one shown in Figure 36 contains a ventilating fan delivering 20 liters of air per minute. The sample passes through a divided cell containing active and inactive Hopcalite. Thermocouples indicate the differences in temperature of the two cells on a scale which is calibrated directly in per cent of carbon monoxide. If the carbon monoxide exceeds 0.02 per cent the meter closes a circuit and sounds an alarm which persists until the meter is manually reset. The model shown in Figure 37 is a completely explosion-proof combustible gas alarm that contains two platinum filaments in a balanced circuit. Catalytic combustion on one of the filaments with a consequent



Courtesy, Arthur H. Thomas Co.

FIGURE 22. DETAILS OF BLACET-LEIGHTON GAS ANALYSIS APPARATUS

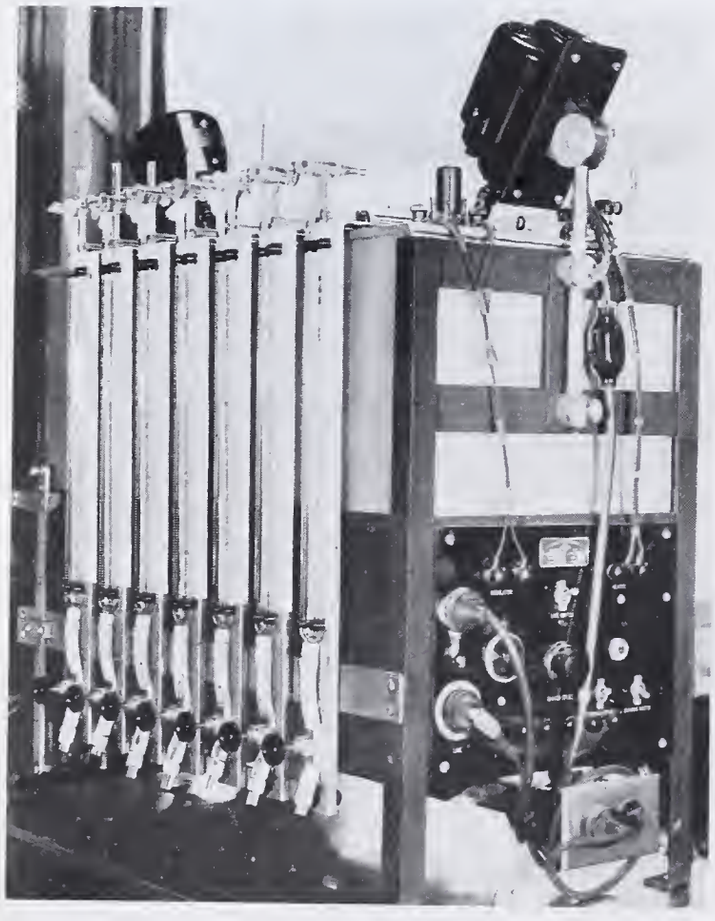


Courtesy, C. N. Frey, Fleischmann Laboratories, Standard Brands, Inc.

FIGURE 24. FLASK-MANOMETER ASSEMBLY FOR BARCROFT-WARBURG APPARATUS

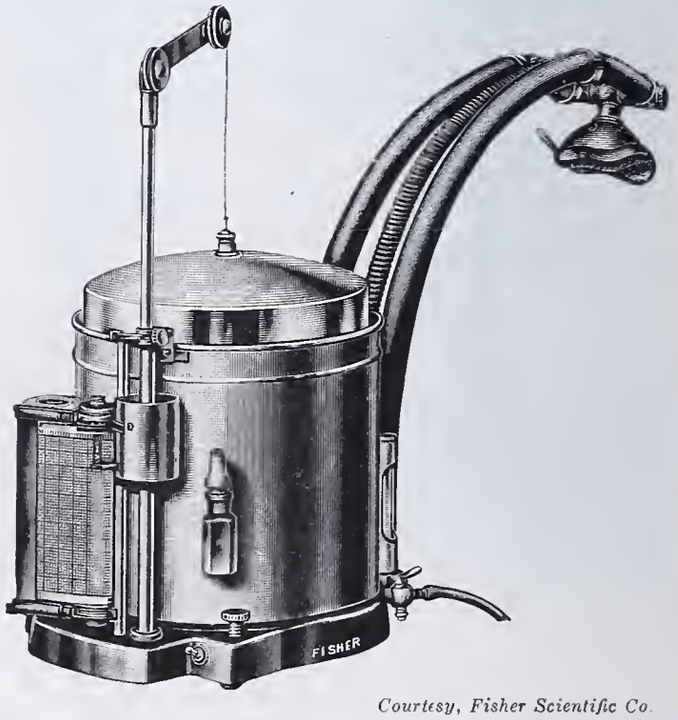
temperature rise unbalances the circuit and indicates directly on the scale calibrated for the gas in question. When the meter reading exceeds a predetermined limit an alarm is sounded which persists until the circuit is manually restored. A nonsparking diaphragm pump furnishes the circulation of the gas.

A very complete classification of the characteristics and applicability of the catalytic conversion technique (*71*) gives details concerning the nature of the gas, range of concentrations, temperature effect per unit per cent of the gas-measuring element, sensitivity, appropriate catalyst, and the field of



Courtesy, C. N. Frey, Fleischmann Laboratories, Standard Brands, Inc.

FIGURE 23. BARCROFT-WARBURG APPARATUS



Courtesy, Fisher Scientific Co.

FIGURE 25. METABOLISM APPARATUS

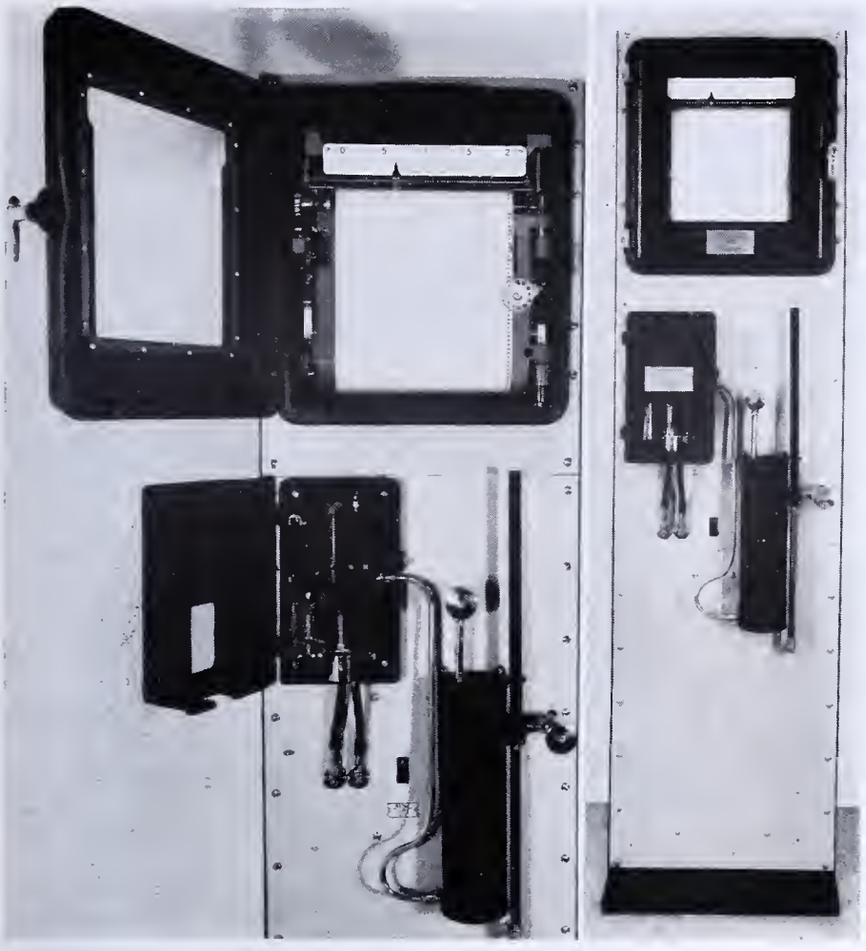
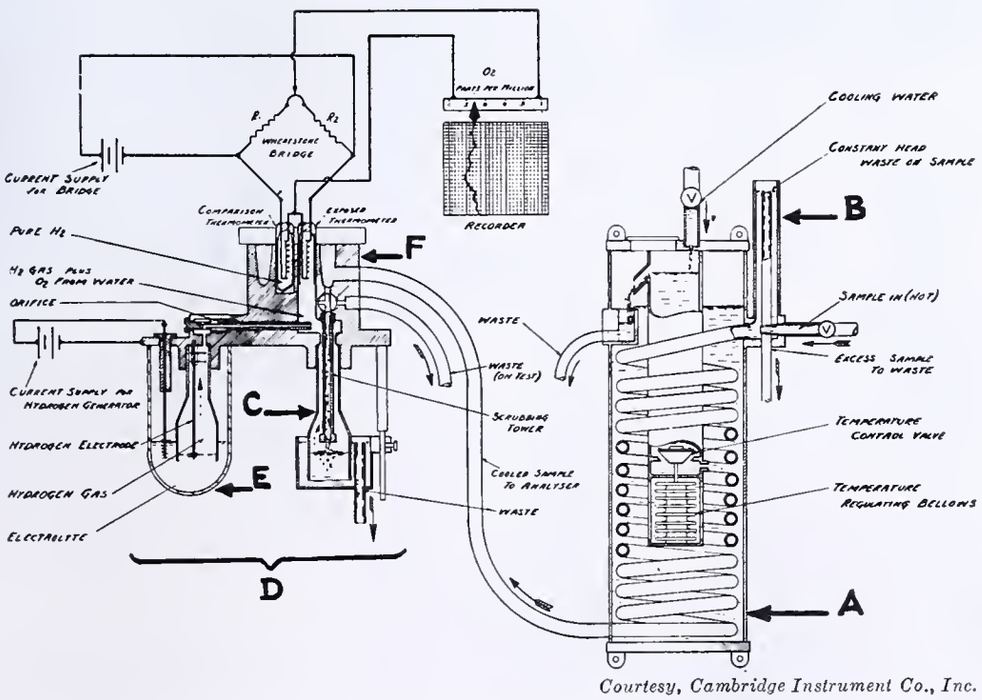


FIGURE 26. DISSOLVED OXYGEN ANALYZER
Courtesy, Cambridge Instrument Co., Inc.

application. In addition to the gases already discussed, ammonia, hydrogen sulfide, methane, hydrogen, and the vapors of benzene and alcohol are included. This list is concerned with actual installations and in principle any system which can be subjected to preliminary chemical treatment can be followed by one of the foregoing instrumental methods.

Optical Methods. Any of the methods discussed below in connection with optical instruments can be applied to the detection or analysis of gases, and this applies for the visible, ultraviolet, and infrared regions of the spectrum. Chlorine, bromine, or iodine vapor is amenable to photometry in the visible, mercury vapor and many hydrocarbons can be detected in vanishingly small amounts in the ultraviolet, and carbon dioxide to the extent of less than 0.0001 per cent can be detected by a characteristic absorption band in the infrared. The use of the interferometer (*63*, *64*) for very exact analyses of gases is discussed in connection with refractive index.

CONCLUSION. The general topic of gas analysis covers an extremely wide range of techniques and particularly from the industrial point of view is in a high state of development. Some of the indirect applications of these methods are startling and in some cases have revived old and almost discarded practices. One illustration which bears this out is difficult to classify but may truly be characterized as an optical method for the analysis of incandescent gas or vapor.



Courtesy, Cambridge Instrument Co., Inc.

FIGURE 27. SCHEMATIC DIAGRAM OF DISSOLVED OXYGEN RECORDER

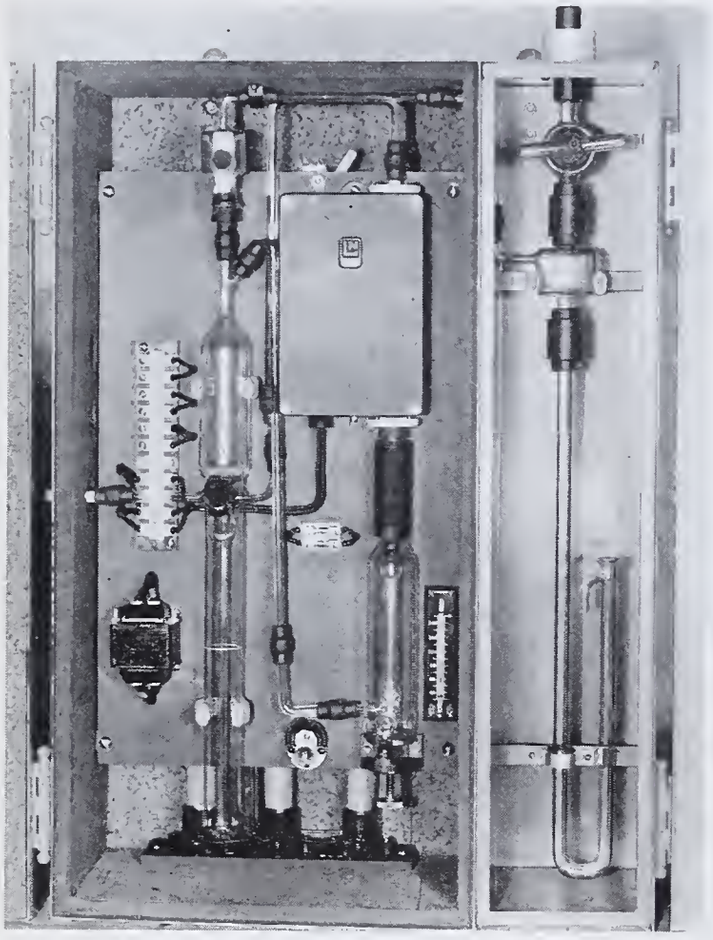
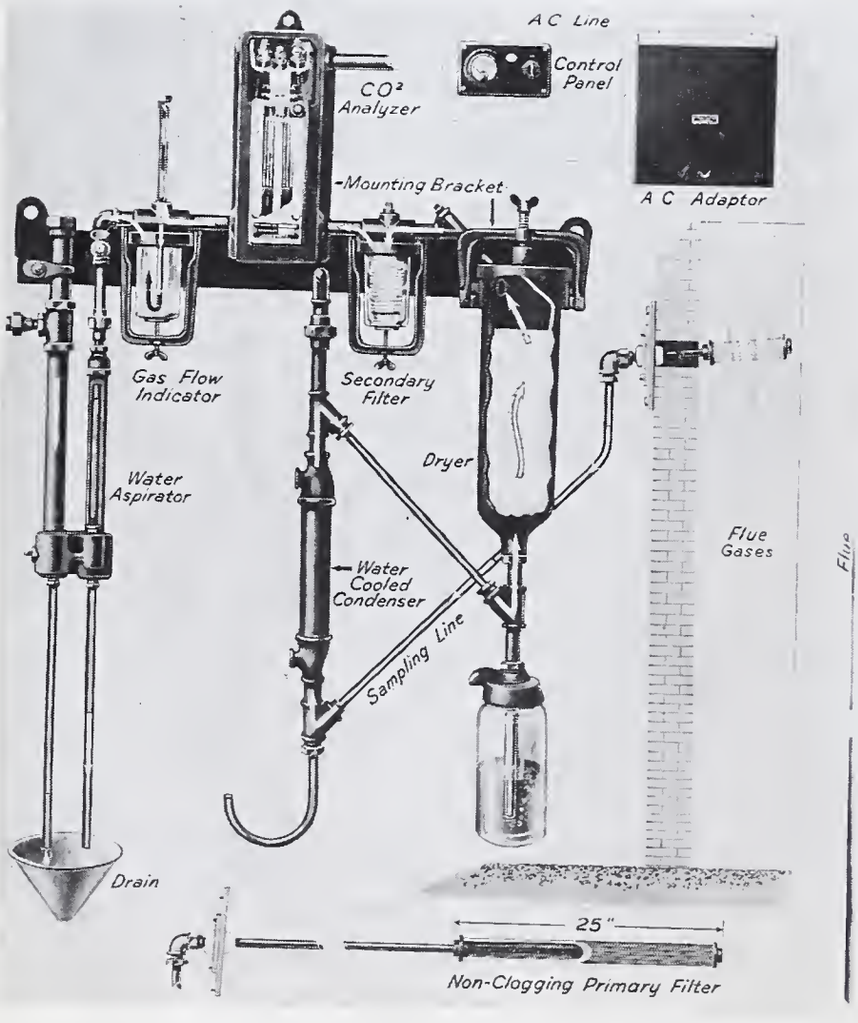


FIGURE 28 (*Left*). MICROMAX CARBON DIOXIDE RECORDER–THERMAL CONDUCTANCE CELL
Courtesy, Leeds & Northrup Co.

Courtesy, Leeds & Northrup Co.

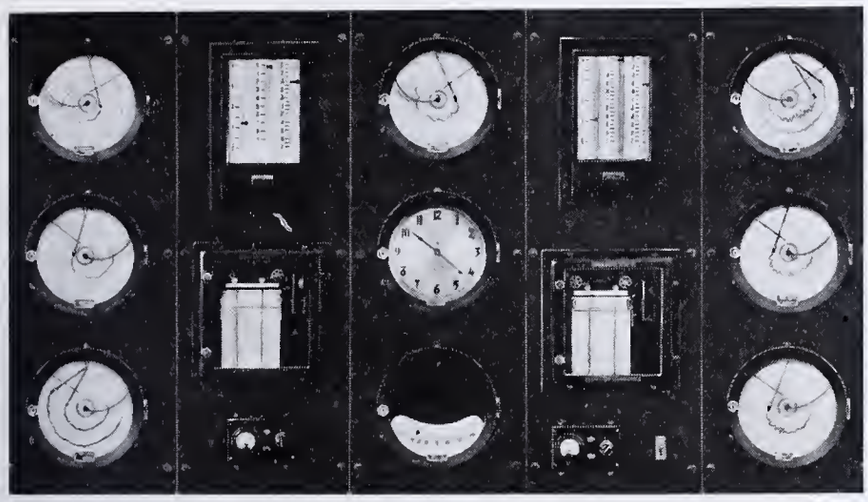
FIGURE 29. MICROMAX CARBON DIOXIDE RECORDER



Figures 38 to 41 show the photoelectric scanning of the flame issuing from a Bessemer converter. Careful spectrographic studies (Figure 38) show that a useful correlation can be obtained between the incandescent gases and the correct blowing time of the converter. The locations of the photocell amplifier, recorder, and the flame target are shown in Figure 39. A complete record is shown in Figure 40, which after appropriate interpretation by the steel master gives a perfectly reliable indication of the condition of the charge.

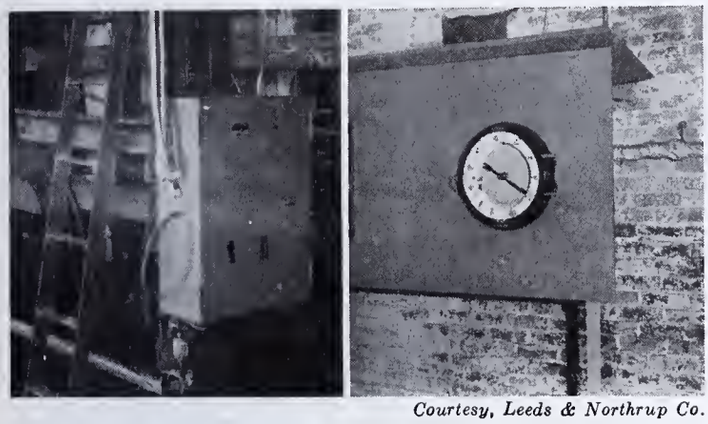
This important investigation has been described in great detail on the basis of countless observations by Work (*303*), and its results have revived great interest in the Bessemer process.

FIGURE 30. SCHEMATIC DIAGRAM OF CARBON DIOXIDE ANALYZER UTILIZING THERMAL CONDUCTANCE PRINCIPLE
Courtesy, Brown Instrument Co.



Courtesy, Brown Instrument Co. and Glidden Co.

FIGURE 31. FLOWMETERS, CARBON DIOXIDE RECORDERS, DRAFT GAGES, AND PREHEATED AIR AND FEED WATER TEMPERATURE RECORDERS ON CONTROL PANEL



Courtesy, Leeds & Northrup Co.

FIGURE 32. SULFUR DIOXIDE RECORDERS

Measuring cell assembly (*left*) detects per cent of sulfur dioxide in continuous sample of flue gas from sulfur burner.
Micromax recorder (*right*) indicates and records per cent sulfur dioxide in flue gas from sulfur burner.

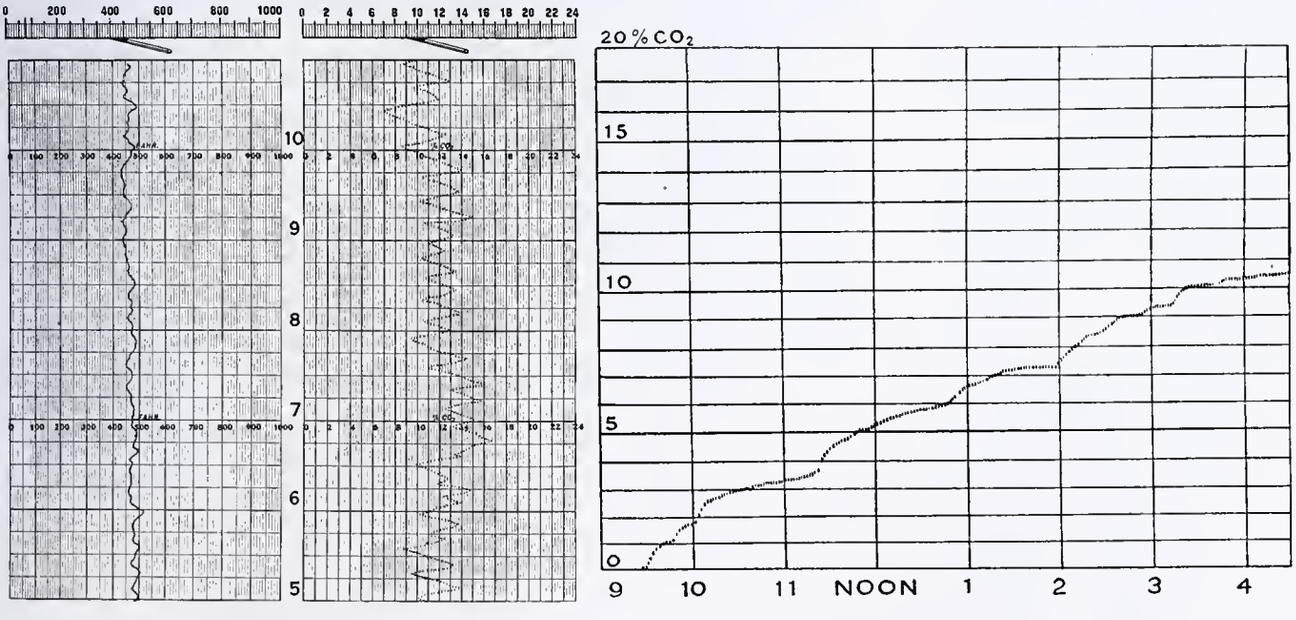
Spectroscopic

An index to the literature on spectrochemical analysis from 1920 to 1939 lists 1446 references (*190*). As the authors of this valuable compilation point out, as late as 1910 it was believed that these methods were of little qualitative value and that quantitative spectrochemical analysis had shown itself as impractical. In addition to about 30 textbooks and manuals on the subject, a number of compilations and bibliographies cover the subject fully (*13*, *34*, *242*, *256*, *257*, *280*, *281*). A very fine approach for the student or beginner is to be found in Brode's text (*30*), which gives a very thorough treatment of the theory, instrumental methods, and applications of spectroscopic methods as well as detailed directions for representative experimental work.

It may suffice here to outline the main features of modern spectroscopic work and to indicate some of the uses which have been made of this very valuable technique.

THEORY. The contributions which spectroscopy has made to our knowledge of the universe range from astrophysics to the energy states in atoms. We are concerned here with the analytical information which they can furnish. The methods are applicable from the infrared to the x-rays.

EMISSION SPECTRA. Under suitable conditions of excitation, atoms can be made to undergo definite electronic transitions which are followed by the emission of characteristic spectral lines. The lines are absolutely characteristic of an element and the certainty associated with this fact makes spectroscopy one of the most reliable means of identification known. The wealth of lines, particularly those due to iron, nickel, tungsten, and many other elements, may cause some confusion, and indeed, skill and experience are required to avoid this complication. (Toxicologists and spectroscopists



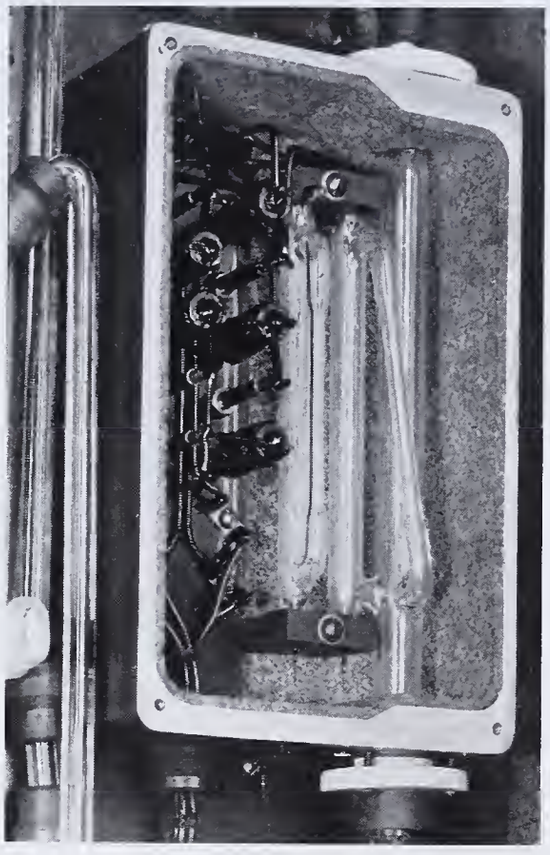
Courtesy, Brown Instrument Co. *"From Dayne's Gas Analysis by Measurement of Thermal Conductivity"*

FIGURE 33. GAS ANALYSIS BY THERMAL CONDUCTANCE MEASUREMENTS

(*Left*) Carbon dioxide analysis from flue gas of a 250-horsepower boiler unit
(*Right*) Respiration of a single housefly (*Diptera domesticus*)

were amused some years ago in New York when one of the prominent iron lines was mistaken for that due to a relatively rare and highly toxic metal.)

The classification of spectral lines has occupied the attention of spectroscopists for many years. One of the finest compilations is the M. I. T. Wavelength Tables (*111*), which contains 109,275 entries giving the wave length, and the intensity in arc, spark, or discharge tube. These tables supersede anything previously done, especially for the analyst, and the details of the job, which employed an automatic computing and recording comparator for determining wave lengths and recording the intensities, are fascinating reading (*110, 113*). The use of tabular compilations of this sort presupposes an accurate measurement of wave length of the observed lines. This is done with an accurate comparator, of which there are several (*197*, Figure 5), and usually in terms of adjoining lines of known wave length. Accurate interpolation can be made by means of dispersion formulas, such as that of Hartmann (*109*). The tables are then consulted and after the responsible element is located it is advisable to seek other known lines of this element on the spectrogram in order to avoid possible confusion with some foreign line. An alternative approach is to photograph a contiguous spectrum, using the suspected substance as a source, and seek identity in the lines. A large number of spectrographically "pure" substances are available for this purpose (*129, 130*).



Courtesy, Leeds & Northrup Co.

FIGURE 34. ALL-GLASS THERMAL CONDUCTANCE CELL

Quantitative spectroscopy may be practiced in a number of ways (*59, 208, 209, 224*). In general, one seeks some connection between the blackening of the plate and the amount of the substance whose spectrum has been excited. A few of the methods may be mentioned; each has its proponents and enthusiasts and volumes have been written on the subject.

1. The use of standard samples containing known amounts of the desired constituent. These and the unknown sample are excited and photographed under identical conditions.
2. The "internal standard" method of Gerlach, in which (*91*) the intensities of the lines in the known and unknown are referred to some common line of an extraneous element which remains unchanged in both spectra.
3. The use of homologous pairs (*91*).

With all these methods the intensity of the lines is measured with a densitometer, examples of which have been described (*197*, Figures 56 to 59); other examples are shown in Figure 48.

INSTRUMENTS AND METHODS. Spectrographs with a prismatic dispersion and dispersion by means of gratings are available in all sizes and types, representative examples of which are shown in Figures 42, 43, and 44. The question of "size" in a spectrograph is determined largely by the nature of the problem. For many purposes, particularly in the nonferrous field, moderate dispersion is satisfactory, but for complex spectra, especially in the ferrous alloys, the highest dispersion is essential. The grating is preferred by many on account of its practically uniform dispersion throughout the spectrum. The relative merits of the two are discussed in standard texts.

Excitation Sources. The spectrum of the sample may be excited in the direct current arc, in the alternating current arc or spark, by the Abriss-Bogen or interrupted arc, and by the acetylene flame (see also *33*). The direct current arc, especially by arcing the sample to exhaustion, affords one of the most sensitive methods. The alternating current arc is more easily controlled, yet retains some of the advantages of the direct current arc. Most of the newer offerings of equipment make provision for all the electrical modes of excitation (Figure 45). An interesting trend is also observable in the number of completely coordinated assemblies which are offered for routine and research work (Figures 46, 47, and 48). It is becoming increasingly apparent to instrument manufacturers that a complete line of instruments for spectrographic analysis is of great advantage and convenience to the customer, and that for continuous routine work he should be furnished with a spectrograph, easily adaptable excitation equipment, densitometer, and other auxiliaries. Most instruments have also been designed with the thought of adapting them to related problems, such as absorption work. This is exemplified by the illustration of spectrophotometry, in which the addition of appropriate accessories converts the instrument into a spectrophotometer.

The numerous illustrations (Figures 49 to 52) given here are intended to illustrate in part correct and convenient modes of installing such apparatus for maximum efficiency (*142, 143*).

A view of the source room in the spectroscopy laboratory at the Dow Chemical Company is shown in Figure 53 (*142, 143, 232–4*).

This fireproof room houses the transformers, motor generators, condensers, resistors, synchronous interrupter, and other auxiliary source equipment necessary for the 35,000-volt alternating current condensed spark, two 25,000-volt alternating current condensed sparks, two 220-volt direct current arcs, and two 2200-volt alternating current arcs.

Each of these excitation sources is wired to a selector panel (Figure 54). One receptacle carries one side of the secondary of each of the sources, the other side being a common ground. The smaller receptacle carries two low-voltage leads for operating a contactor for each source. By means of this panel any source can be made available at any one of the four spectrographs located around the source room.

A view of the plate-interpretation room (Figure 55) shows the Hilger nonrecording microphotometer and the calculating board, used to convert microphotometer readings to per cent of the element in question.

Figure 56 is a view of one of the Bausch & Lomb medium quartz spectrographs. In addition to the spectrograph there is

to be seen the outlet panel on which are mounted the control switch, warning lights, and double range ammeter, in addition to the power outlets.

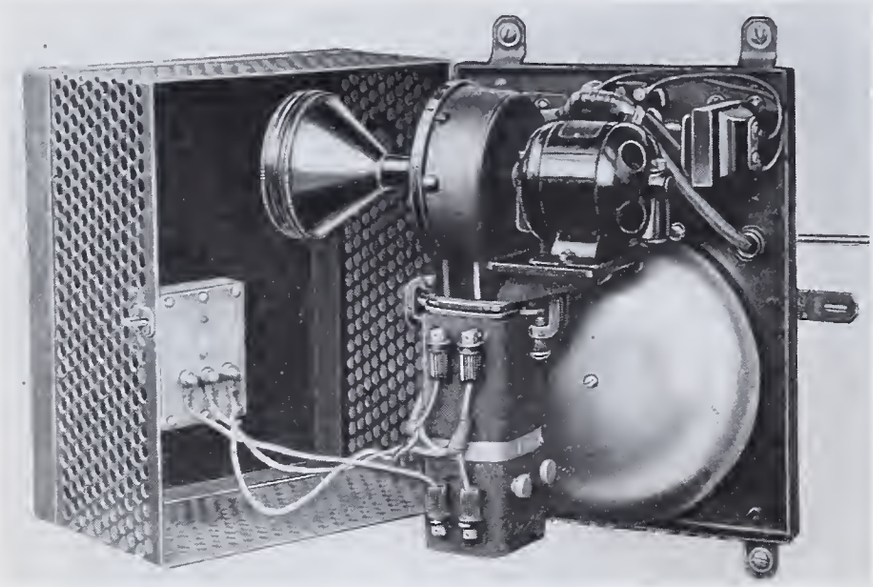
A similar outlet panel is located above the source tables before the other Bausch & Lomb medium quartz instrument, the Gaertner quartz Littrow, and the Cenco Replica grating instrument.

Applications. For the countless applications of emission spectroscopy the reader must be referred to the copious references in the compilations quoted. They cover every conceivable field of analysis, from the examination of alloys, works of art, biological fluids, foodstuffs, toxicological (*37*, *38*) and forensic specimens, to products of combustion, gases in internal combustion engines, fuels, glass, plants, and water analysis.

Precision, Rapidity, and Costs. It is not unnatural for the beginner or prospective spectroscopist to raise questions of precision, rapidity, and costs. A glance at any spectrographic laboratory will tell him that this is very expensive and elaborate equipment and although the wide range of applicability may be evident, he is interested in a definite answer to these questions. Since spectroscopic methods are among the most delicate which we have, and combine with a high degree of certainty and reliability, it is worth noting at the outset that the precision even for minute traces is rarely less than 10 per cent; and with the very best resources of densitometry

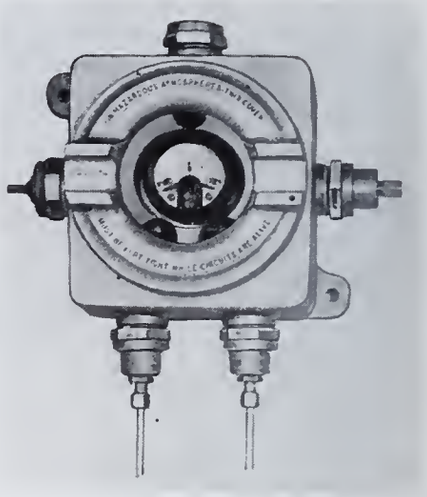
Courtesy, Mine Safety Appliances Co.

Figure 35. (*Upper Left and Lower Right*) Explosimeter. (*Center*) Combustible Gas Indicator. (*Lower Left*) Gas Indicator. (*Upper Right*) Carbon Monoxide Indicator



Courtesy, Mine Safety Appliances Co.

FIGURE 36. CARBON MONOXIDE ALARM AND VENTILATION CONTROL



Courtesy, Mine Safety Appliances Co.

FIGURE 37. EXPLOSION-PROOF COMBUSTIBLE GAS ALARM

and under carefully controlled conditions may be made as high as 2 or 3 per cent. Concerning the rapidity of the methods much has been said and published, but in the early days of quantitative spectroscopy it was shown by Meggers (*190*) that a series of analyses which required a full day's work by conventional chemical methods could be accomplished in less than 2 hours spectrographically and with no particular sacrifice in precision. The rapidity of the method must be gaged on the assumption of very careful preliminary development of the method and operating conditions. It is essentially in routine examinations that the economy in time becomes more and more apparent. In many procedures by the judicious apportioning of the work it has been possible to reduce quantitative analyses to a few man-minutes per determination.

Costs must be appraised on the same basis, especially in view of the high initial cost of the equipment, but here again experience has shown on numerous occasions that the costs can be brought even below those of conventional methods. Again in routine work it is apparent that cost and speed are interrelated and an increase in the former implies a certain reduction in the latter.

The present enormous industrial activity will undoubtedly produce new and startling figures bearing on these questions.

Absorption Spectroscopy–Spectrophotometry

Spectrophotometry is concerned with measurement of the amount of light absorbed or reflected at each wave length through the spectrum. It may be applied to the visible, ultraviolet, or the infrared. It will be more convenient to discuss the latter separately, although the laws applying to it are the same.

The law applying to the absorption of light at each wave length is that of Lambert and Beer.

The transmission, T, is defined as the ratio of the emergent intensity, I, to the incident intensity, I_0. For a solution containing a light-absorbing species of concentration c, in a layer of thickness t, we have the relationship:

$$I = I_0 \times 10^{-kct} \qquad (1)$$

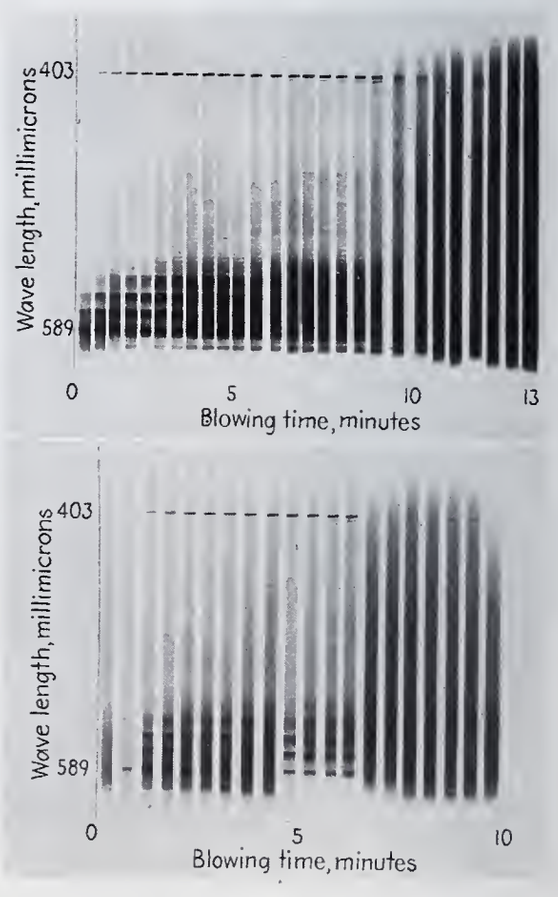
from which we may write

$$-\log I/I_0 = kct = -\log T \qquad (2)$$

or

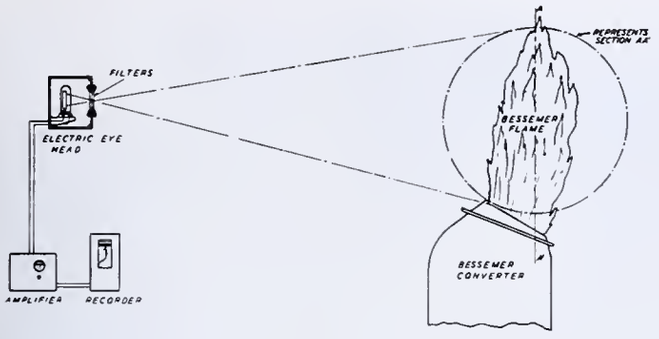
$$\log I_0/I = kct = E \qquad (3)$$

where E is the extinction, E/t is the extinction coefficient, and k is the molar extinction coefficient, provided c is expressed in moles per liter. There is no particular uniformity in terminology or symbols in this field and it is still necessary for investigators to report the meaning of the symbols which they employ and the units in which they are expressed. Some investigators have used the term density, D, instead of extinction, E. The use of E conforms more nearly with European practice and D seems to have



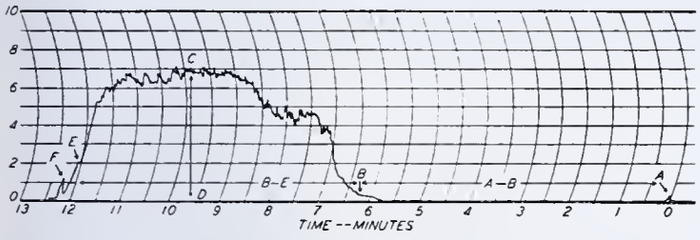
Courtesy, H. K. Work, Jones and Laughlin Steel Corp.

FIGURE 38. SPECTROGRAMS OF BESSEMER FLAME AS A FUNCTION OF BLOWING TIME



Courtesy, H. K. Work, Jones and Loughlin Steel Corp.

FIGURE 39. SCHEMATIC VIEW OF PHOTOELECTRIC BESSEMER FLAME SCANNING



Courtesy, H. K. Work, Jones and Loughlin Steel Corp.

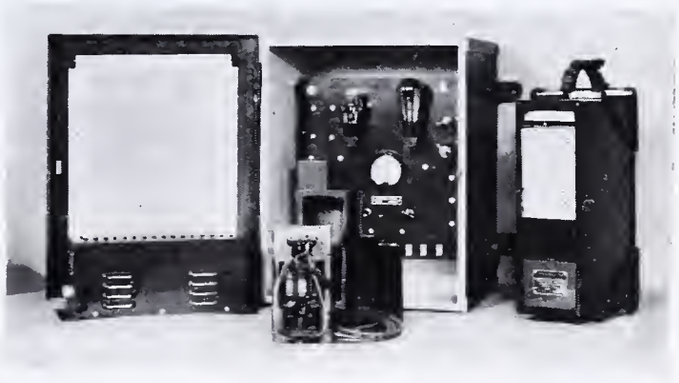
FIGURE 40. AUTOMATIC RECORD OF BESSEMER BLOW

been borrowed from the field of photographic densitometry, where it is used to give an exact expression for the blackening of a plate.

The foregoing discussion of extinction and extinction coefficients implies a definite wave length, λ. Indeed, the variation of extinction with wave length is the only fundamental way of expressing the "color" of a light-absorbing substance and a curve relating extinction to wave length gives us the absorption spectrum or characteristic color curve. To be sure, in most measurements the transmission is measured directly and the extinction or extinction coefficient must be computed from the transmission data. It can be shown that if one plots log extinction as a function of the wave length, such curves will be still more useful from the point of view of identifying a colored substance. Such curves are independent of the concentration and thickness and can be superimposed by a shift along the ordinate (log E) axis. This is shown very nicely by Figure 57 (upper)[1], which shows the log extinction curves for a dye notorious for its deviation from Beer's law. The curves are taken at six different concentrations (1.25, 2.5, 5, 10, 20, and 40 mg. per liter), and should be directly superimposable by shifting them along the ordinate axis if the typical "color" is independent of concentration. That this is not the case for this substance is shown in the lower graph, which illustrates the failure of Beer's law except at a pivotal wave length of approximately 552 millimicrons. This shows the Beer's law can be applied with confidence at this wave length but would be highly in error at others, particularly at the longer wave lengths.

Very frequently elaborate series of transmission curves are presented to illustrate changes in the absorption of a substance, but they are almost useless in that form for any accurate appraisal of the changes unless they are recalculated to the log E basis. To be sure, the point-by-point recalcula-

[1] The writer is greatly indebted to W. A. Shurcliff of the Calco Chemical Division, American Cyanamid Company, for the preparation of the curves in Figure 57, to illustrate this important principle.

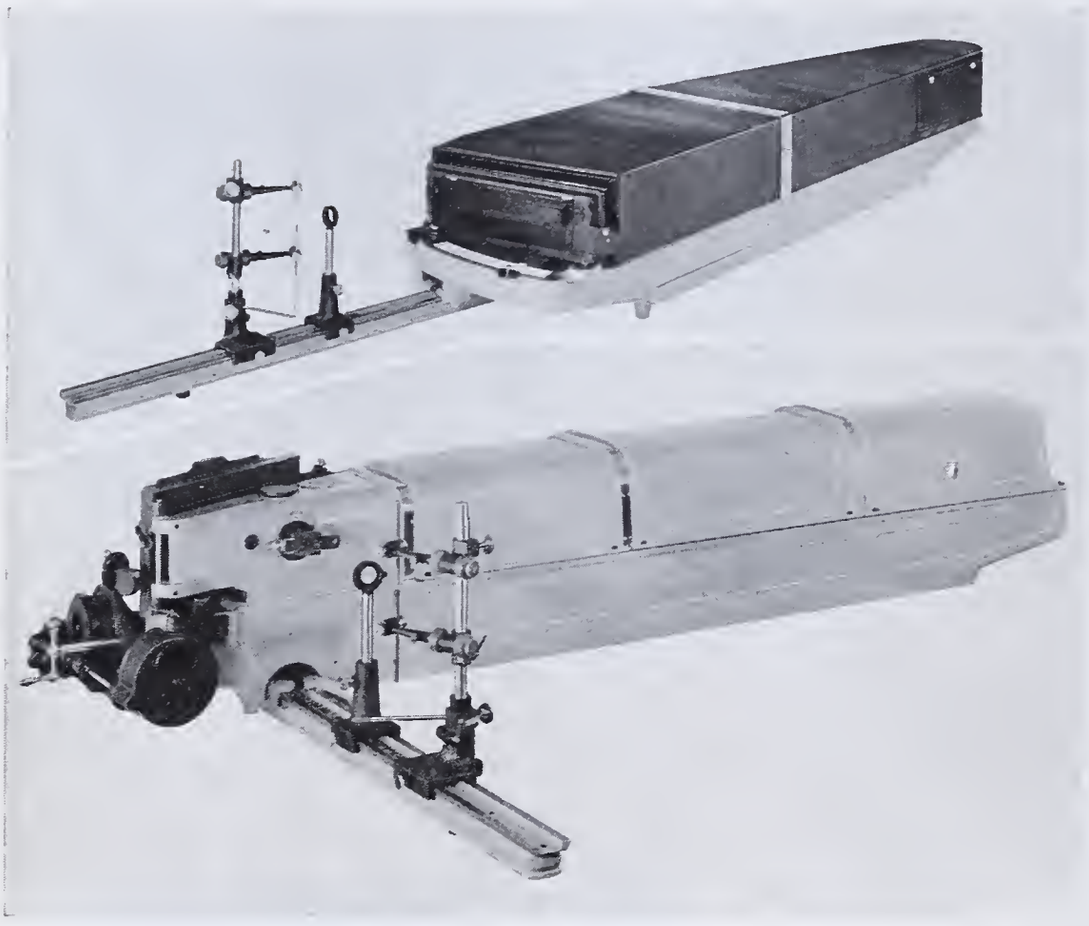


Courtesy, H. K. Work, Jones and Laughlin Steel Corp.

FIGURE 41. PHOTOCELL HEAD, AMPLIFIER, AND RECORDER FOR CONTROL OF BESSEMER STEEL MAKING

Courtesy, Bausch & Lomb Optical Co.

FIGURE 42. SPECTROGRAPH



Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.

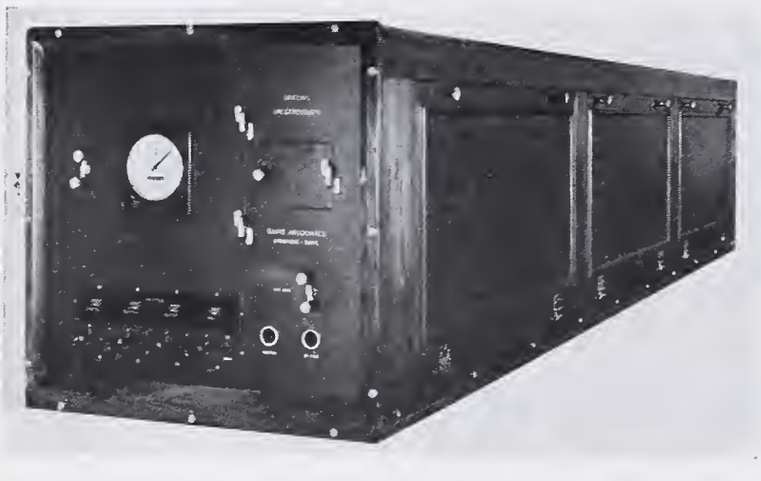
FIGURE 43. (*Upper*) TECHNICAL GRATING SPECTROGRAPH. (*Lower*) FULLY AUTOMATIC LARGE QUARTZ AND GLASS SPECTROGRAPH

tion is laborious, but in the case of recording spectrophotometers the labor is completely eliminated by the introduction of a log log $1/T$ cam (Figure 58) with which curves similar to Figure 57 are obtained.

Analytical interest resides in the fact that for systems obeying Beer's law a plot of extinction against concentration is linear. In seeking the conditions under which this useful relationship may be obtained the importance of the log E curves is once more evident, in that if the curves are completely superimposable Beer's law will hold at any wave length. If there is a common point, as in Figure 57 (lower), that wave length alone will be satisfactory. In the former case the principal advantage lies in the fact that one has complete freedom of choice, which may be very useful if other light-absorbing species are present.

FIGURE 45. CONTROL PANEL FOR SPECTROGRAPH EXCITATION EQUIPMENT

Courtesy, Baird Associates

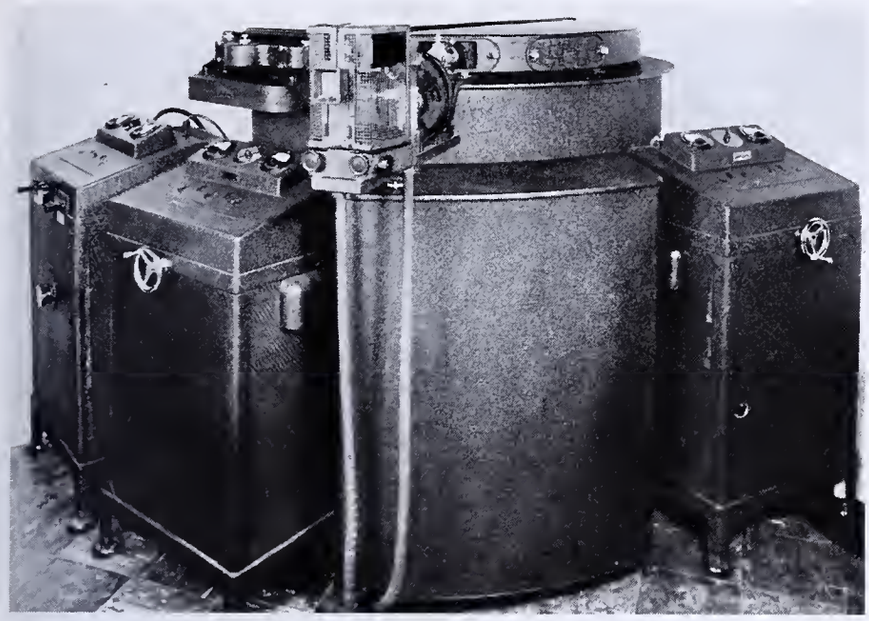


Courtesy, Baird Associates

FIGURE 44. THREE-METER GRATING SPECTROGRAPH

The analysis of mixtures is spectrophotometrically feasible, since the extinctions are additive and the method merely presupposes that there is no interaction between the two light-absorbing entities. The theory of this and the other topics discussed here is covered in standard references (*30, 114*).

INSTRUMENTS AND METHODS. *Visible Spectrum.* In all spectrophotometers a source of continuous radiation is spectrally resolved by means of a direct-reading spectrometer or monochromator, either before or after the photometric matching.



Courtesy, Harry W. Dietert Co.

FIGURE 46. SPECTROGRAPH ON METAL BASE WITH THREE SOURCE UNITS

given by the effective opening of the sector and can be measured mechanically with a high degree of precision. An example of this class is shown in Figure 62.

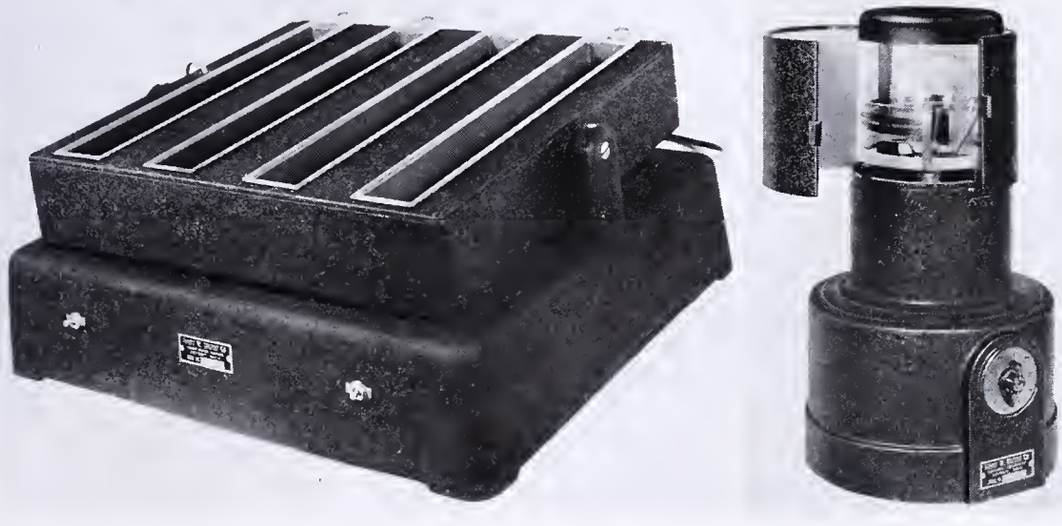
3. Photoelectric Spectrophotometers. In these the relative intensity of the measuring and reference beams are measured photoelectrically following the spectral resolution of the beams (Figure 63). Two manually operated examples of this class have been described (*197*, Figures 63 to 66). A completely automatic instrument developed some years ago by Hardy is the General Electric color analyzer (Figure 64). This well-known instrument has been described in several places and many important researches have been based on its use. It operates on a strictly null principle in which the unknown and comparison beams are rapidly intercompared and a photocell-thyratron unit controls a motor which continuously rebalances the system. A complete spectrum may be recorded in from 2.5 to 5 minutes. Numerous attachments are available in the form of computed cams (Figure 58), whereby the results will be plotted as per cent transmission or reflectance, extinction or log extinction. A somewhat more complex attachment is available for computing trichromatic coefficients directly.

1. The Polarization Type. In this class the measuring beam and the comparison beam are matched for equal intensity by reducing the intensity of the comparison beam by a polarizing mechanism (*294*). The decrease in intensity is a trigonometric function of the angle of rotation of the polarizing element. It is specifically in terms of two match points. The transmission is given by $T = \cot^2 \theta \times \tan^2 \varphi$ where θ is the larger angle; or if sample and solvent are interchanged and the measurement is repeated, the transmission is given by $T = \cot \theta \times \tan \varphi$. Examples of this class are the Königs-Marten, Bausch & Lomb (Figures 59, 60, and 61), and Nutting (Hilger). As a rule the scale attached to the polarizer head is calibrated to read the angle in degrees, another portion is engraved in transmission, and a third in extinction values. This is a widely used and time-honored type. The only drawback is the caution which must be exercised in measuring the reflectance of samples which might give rise to polarization effects due to surface structure or other causes.

2. Sector Type. In this instrument the photometric matching is achieved by varying the aperture of a rapidly rotating sector while it is in rapid motion. The rotational rate of the sector is high enough to eliminate any perceptible flicker. The scale usually reads directly in transmission values which are really

These instruments are all to be regarded as the fundamental standards for expressing "color" and are capable of high precision in the hands of an experienced operator (*108*, *109*). They have been used very extensively in chemical analysis. They are expensive by virtue of their elegance and precision, and for many analytical purposes some of the compromise instruments which are discussed below will do just as well. However, in any extended program of work one has constant and repeated need for recourse to this important instrument. The photoelectric type is not necessarily more accurate but does have unusual advantages in the extreme ends of the visible spectrum where the visibility function is poor.

Ultraviolet. Most of the information in this region can be obtained only by photographic or photoelectric means. Any spectrograph may be fitted with means of splitting the incident light into two beams, in one of which a sample is placed and in the other some intensity weakening mechanism is introduced. A common form of intensity reducer takes the



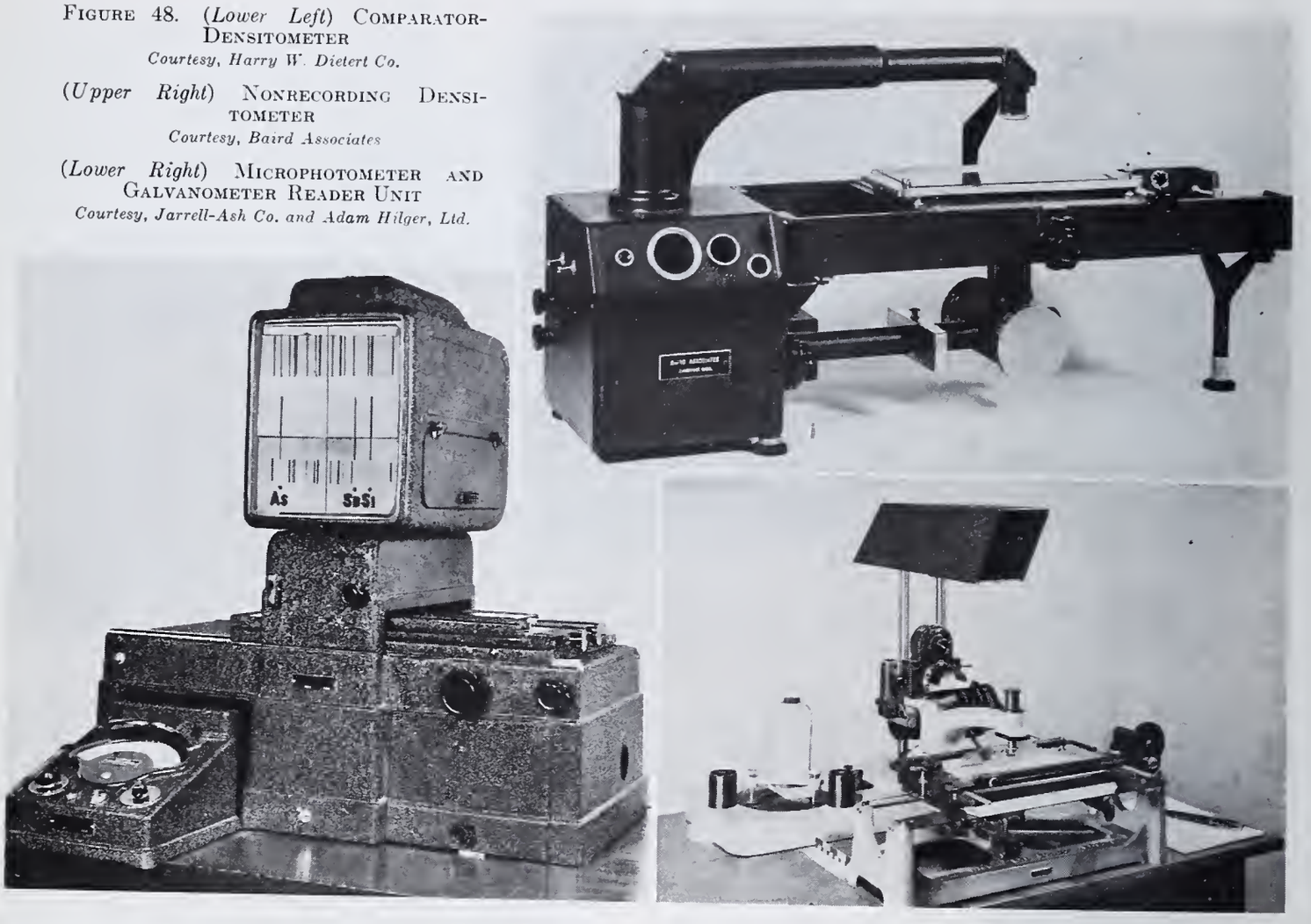
Courtesy, Harry W. Dietert Co.

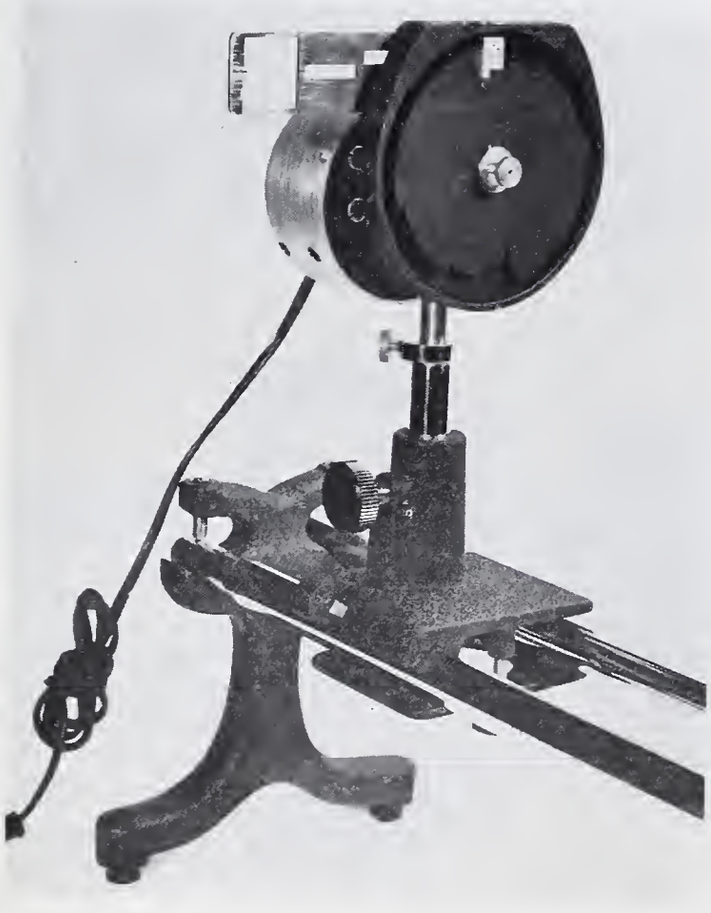
FIGURE 47. (*Left*) ARL-DIETERT FILM-DEVELOPING MACHINE. (*Right*) ARL-DIETERT FILM INFRARED FILM DRYER

FIGURE 48. (*Lower Left*) COMPARATOR-DENSITOMETER
Courtesy, Harry W. Dietert Co.

(*Upper Right*) NONRECORDING DENSITOMETER
Courtesy, Baird Associates

(*Lower Right*) MICROPHOTOMETER AND GALVANOMETER READER UNIT
Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.





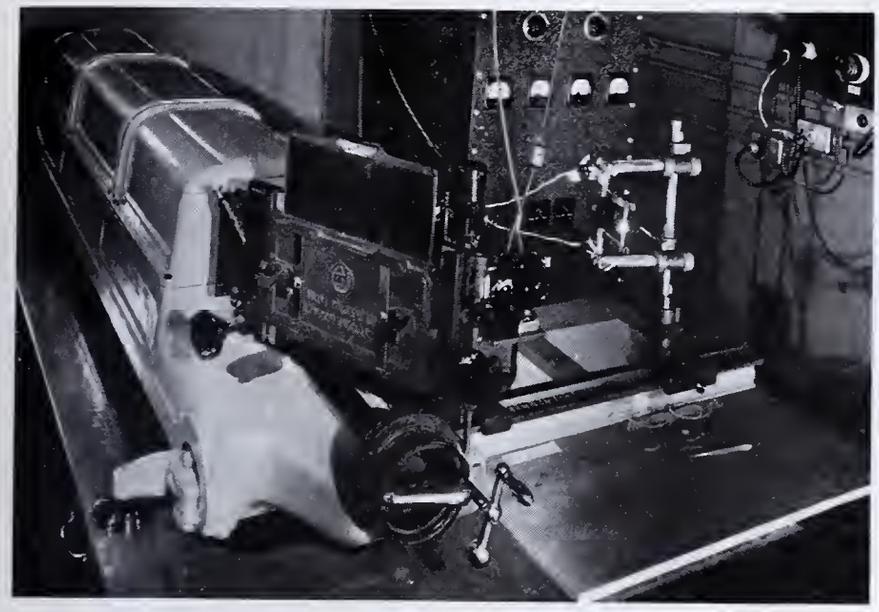
Courtesy, Baird Associates

FIGURE 49. STEP-SECTOR ATTACHMENT FOR SPECTROGRAPH

form of a rotating sector (Figure 65), and another of an adjustable diaphragm (Figure 66). If these pairs of spectra are photographed side by side and this process is repeated for an extended series of sector or diaphragm settings, the absorption curve is obtained from the spectrogram by noting the wave length at which intensity matches can be found (Figures 67 and 68). For careful work a series of spectra at various intensities can be recorded to calibrate the photographic plate itself, since, as is well known, the blackening-intensity relationship of the photographic plate is complex (*109*). An iron arc or an underwater spark may be used as the light source for this type of work, but a perfectly continuous source such as the hydrogen discharge tube is preferable. Measurements of this type are exact and furnish accurate extinction values, but for some semiquantitative results it is often feasible to make a single measurement through a solution and to record the intensities with a densitometer. A comparison record must be obtained for the source alone in order to lump or integrate the combined effects of energy distribution in the source and the characteristics of the plate.

Photoelectric instruments of two classes have been developed, manual and photoelectric. Precise examples of the former have been described by Hogness (*133*) and von Halban (*294*) and a recent addition to this class is illustrated by Figures 69 and 70. Recording instruments have been described by Harrison (*112*) and Brode (*30*).

The experimental difficulties attending the construction and use of these instruments are far greater than the corre-



Courtesy, Jarrell-Ash Co., Adam Hilger, Ltd., and Eastman Kodak Co.

FIGURE 50. FULLY AUTOMATIC LARGE QUARTZ AND GLASS SPECTROGRAPH

light in this region, together with the related information from dielectric measurements and Raman spectra, has contributed enormously to our knowledge of molecular structure. The great amount of data which has been accumulated primarily for this purpose is beginning to be used and appreciated for its analytical value and recently it has turned out that despite great experimental difficulties and complications this technique will have an almost revolutionary effect on the analytical chemistry of the future. Some of the finest resources of modern research have been directed precisely to this end.

A negligible portion of the infrared is accessible by photographic technique and it is necessary to measure the absorption in this region by receiving the spectrally resolved radiation on the surface of a sensitive thermopile. Until very recently it has been necessary to study absorption in this region by means of point-to-point measurements of thermopile-galvanometer readings throughout the spectrum. One of the useful characteristics of absorption in this region is the distinctive nature of many of the vibrational bands and their unmistakable association with definite atomic linkages—for example, in Table II, reproduced from Brode (*30*), definite frequencies can be associated with the indicated functional groups.

It is characteristic also that, especially in homologous series, the frequency does not shift greatly, but there may be very considerable intensity variations. For most analytical purposes one is not concerned with the subtler differences in band structure but solely with the general nature and frequency allocation.

TABLE II. FREQUENCY VALUES FOR INFRARED ABSORPTION BANDS DUE TO INDICATED LINKAGES

Functional Group	Frequency, ν' $Cm.^{-1}$	Functional Group	Frequency, ν' $Cm.^{-1}$
≡C—H	2910	—S—H	2500
≡C—C≡	990	=N—H	3300
=C=C=	1630	≡C—I	500
—C≡C—	2200	≡C—B	560
=C=O (ketones)	1722	≡C—Cl	650
=C=O (acid)	1660	—C≡N	2250
≡C—N=	1035	—N≡C	2150
≡C—O—	1034	=N—O—	1003
—O—H	3380	—NH—NH—	3000

sponding problem in the visible, but so much valuable information is obtainable in the ultraviolet that the problem has rightly engaged the interest of many experts.

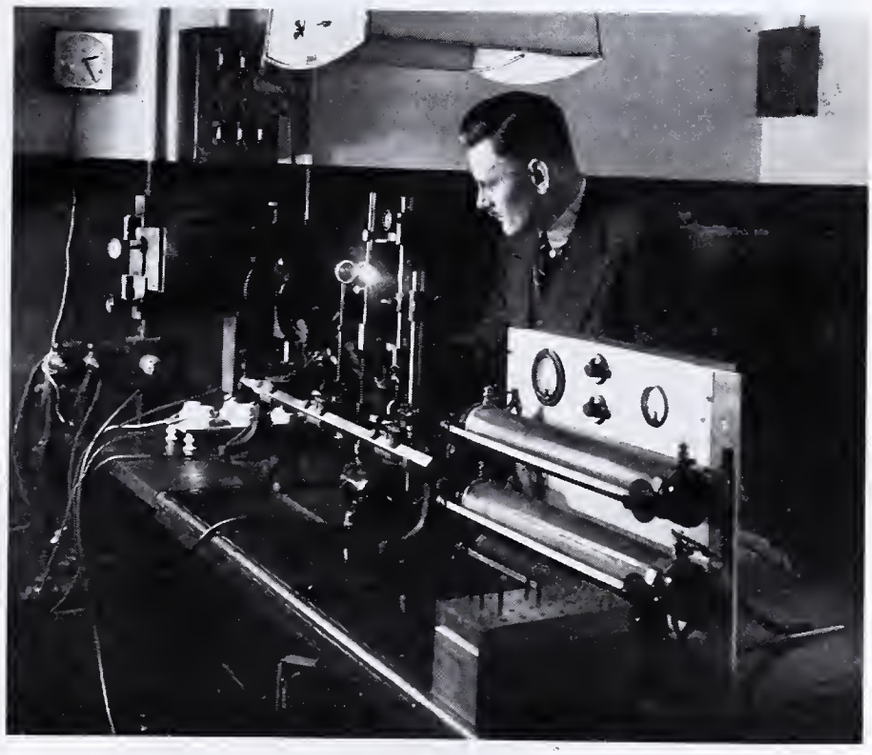
Other examples are illustrated in Figures 71 and 72.

APPLICATIONS. A recent compilation (*288*) lists 866 references to spectrophotometric investigations, covering analytical problems in every imaginable field: inorganic, organic, biological, metallurgical, and industrial (*191*, *202*). A few more or less generic or typical problems (*4*) include:

1. Characteristic identification (dyes)
2. Determination of mixtures
3. Study of equilibria (pH with indicators)
4. Confirmation of structure as a liaison medium between analysis and synthesis, an excellent recent example of which is the case of vitamin B_1 (*230*).

Infrared

The fundamental information which has been derived from the absorption of



Courtesy, Bell Telephone Laboratories

FIGURE 51. ANALYTICAL APPLICATION OF SPECTROGRAPH

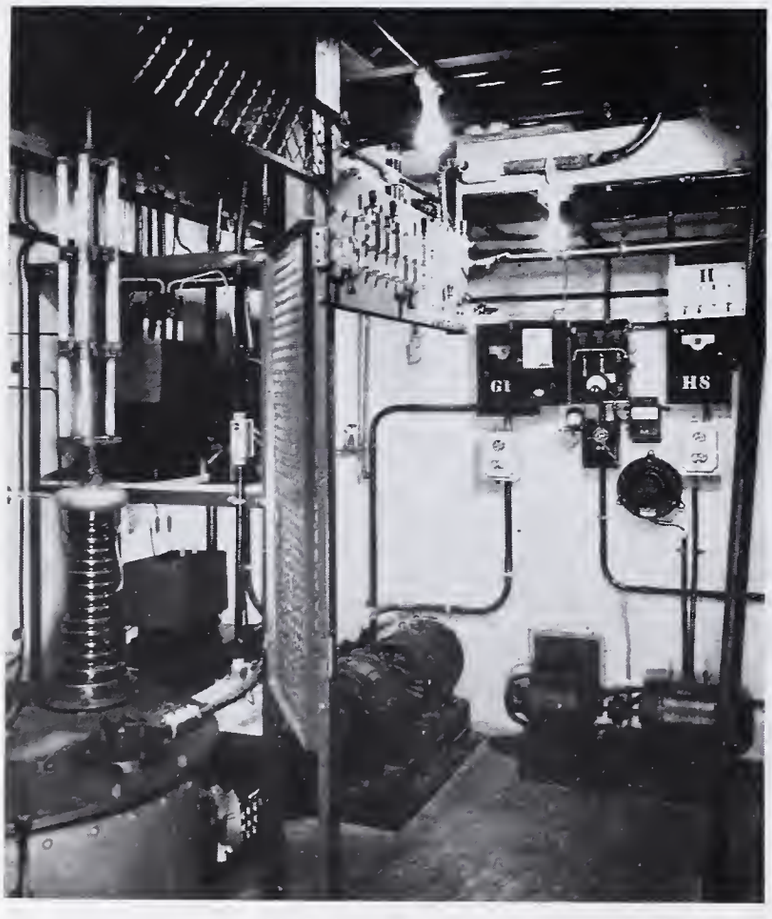
FIGURE 52. (*Upper Left*) INDUSTRIAL INSTALLATION USING LARGE LITTROW QUARTZ SPECTROGRAPH

Showing excitation stand and fume hood and controls. Utilizes direct current arc, high-voltage spark, and modified Feussner spark and disruptive spark

(*Upper Right*) VISUAL COMPARISON AND ANNOTATION OF PLATES

(*Lower Left*) DIRECT-READING MICROPHOTOMETER AND AMPLIFIER FOR SPECTROGRAMS

Courtesy, Lucius Pitkin, Inc.



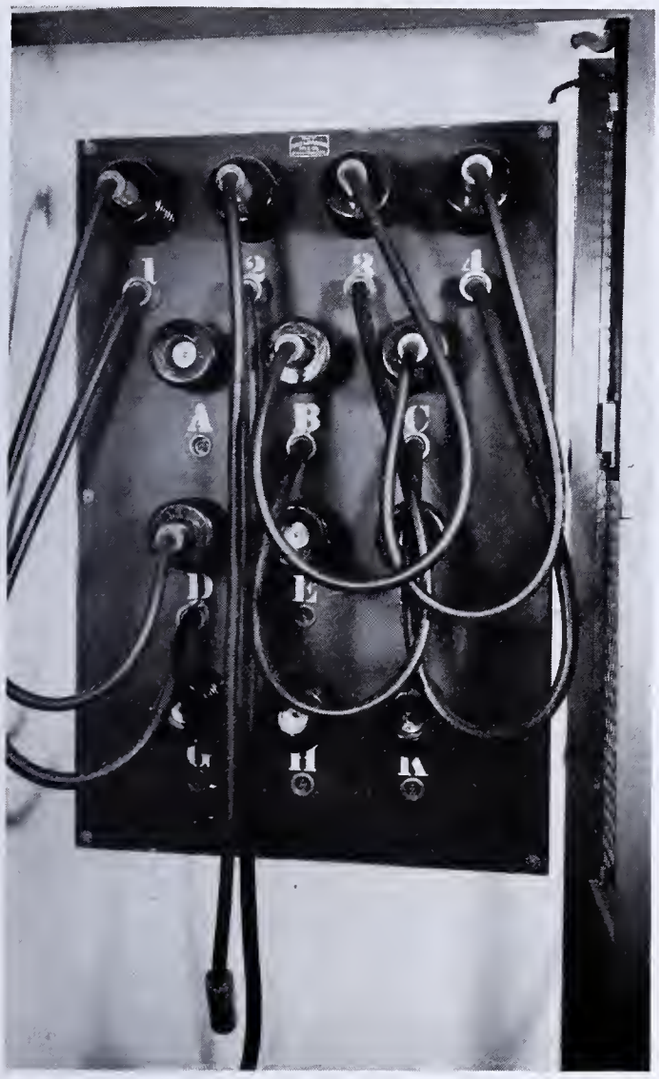
INSTRUMENTS AND METHODS. A typical arrangement for this class of measurement is illustrated in Figure 73.

Radiation from a Glo-bar source is passed to a series of mirrors which focus radiation on a narrow slit. The radiation proceeding from this slit is rendered parallel and strikes a rock-salt prism arranged in Littrow mounting. The dispersed radiation is reflected from the Littrow mirror, sent once more through the prism, and ultimately brought to focus on a thermocouple connected to a highly sensitive galvanometer.

So far the arrangement is representative of most infrared setups. Most earlier attempts to make the

FIGURE 53. SOURCE ROOM, SPECTROGRAPHIC LABORATORY

Courtesy, Dow Chemical Co.



Courtesy, Dow Chemical Co.

FIGURE 54. SELECTOR PANEL FOR EXCITATION SOURCES IN SPECTROGRAPHIC LABORATORY

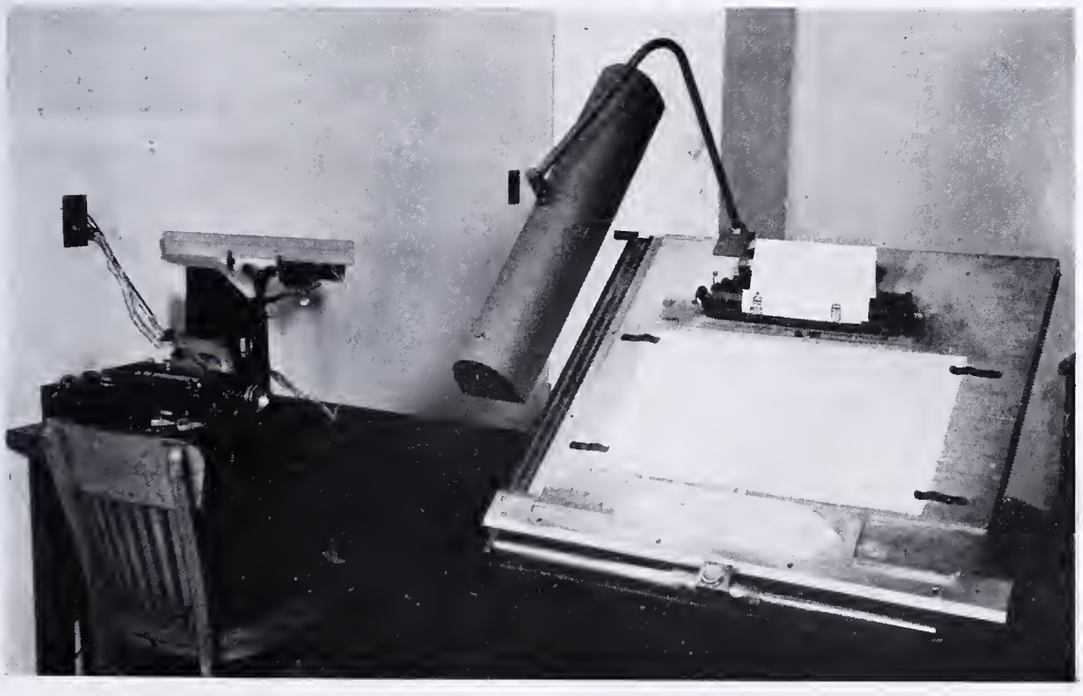
assembly automatically recording utilized photographic recording of the galvanometer deflections. The improvements in this particular installation provide electronic means for following galvanometer deflections.

A motor in the recorder unit slowly moves the dispersion train through a worm gear and sector, as shown in Figure 73, so that the entire spectrum from 2μ to 11μ is slowly swept across the exit slit. The same motor drives a drum in the recorder unit on which a large piece of graph paper is mounted. The deflection of the light beam from the galvanometer mirror is detected by means of a photoelectric follow-up mechanism: a photocell unit motor-driven in such a manner that it constantly "chases" the light beam. This unit also carries a pen or stylus which draws a line on the recorder paper. Thus a continuous curve is drawn, and is visible at all times to the operator, relating energy to wave length. If an absorbing substance (sample) is placed in the optical path of the spectrometer, the regions of characteristic absorption will appear in the record and simple computations from the record will reduce this curve to an equal energy base.

The data for more than 600 key organic compounds have been recorded with this instrument and even at this stage of progress give a quick and reliable answer to many important problems. Arrangements have been made for the detailed description and list of uses of this fine installation to appear in the ANALYTICAL EDITION. Some of its uses are illustrated in Figures 74 to 78.

Figures 79 and 80 show two views of an automatic, recording infrared spectrometer with optical path enclosed in an air-tight case of Dowmetal. Although similar to a previously described instrument (*304*), it has a number of improvements.

It is equipped with exchangeable 60° prisms of rock salt and lithium fluoride which operate in a Littrow mounting. All controls of the apparatus are located at one end of the spectrograph within easy reach of a seated operator. Widths of the slits can be varied by turning a crank, wave lengths can be determined from



Courtesy, Dow Chemical Co.

FIGURE 55. PLATE-INTERPRETATION ROOM IN SPECTROGRAPHIC LABORATORY, WITH DENSITOMETER AND COMPUTING BOARD

Courtesy, Dow Chemical Co.

FIGURE 56. QUARTZ SPECTROGRAPH IN SPECTROGRAPHIC LABORATORY

a revolution counter, drive speeds can be easily changed by rearranging a belt and pulley system, and a radiation shutter can be opened or closed by turning a knob on the switch panel.

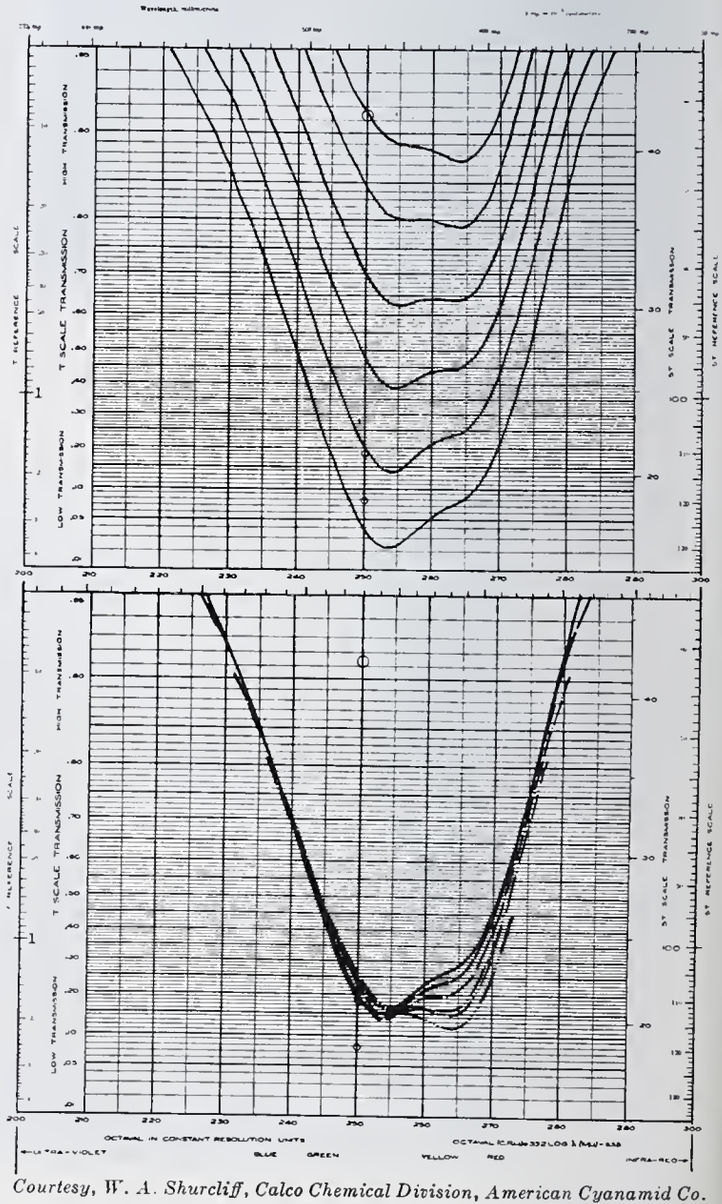
The galvanometer deflections can be followed visually on the ground-glass scale while being photographically recorded on the drum camera which is within the operator's reach. A separate air-tight case contains the Globar source and focusing mirror. This unit is made movable, so that all air space between it and the sample cells, which are of different thicknesses, can be displaced.

Typical results obtained with this type of instrument are shown in Figure 3 of Wright's paper (*304*).

Very useful results have been obtained without the use of a spectrometer by using a scheme proposed by Pfund. As used for the estimation of carbon dioxide, radiation from a hot carbon dioxide source (Bunsen burner) is passed through a cell to a receiver (thermopile) immersed in carbon dioxide. In the absence of carbon dioxide in the absorption tube the receiver heats up as a result of absorption of the radiation selectively emitted by the source. In the presence of carbon dioxide in the absorption tube, the heating of the receiver is diminished. This method should be applicable to all gases which can be caused to emit the infrared radiation which they absorb.

APPLICATIONS. It can be inferred from Table II that the number of compounds which can be investigated by this powerful tool is almost unlimited and the systematic accumulation of data which is now proceeding rapidly in many places will eventually lead to a convenient form of classification from which direct analytical information may be deduced with ease. One is not to infer that we are only now in a position to learn something about the infrared; that process has been going on for years, but with the increasing realization of the distinctive analytical use the classification and interpretation will be directed to those uses.

There is a high degree of specificity and certainty about these measurements; they can be extraordinarily sensitive as well—for example, it is possible to determine accurately as little as one part of water in a million parts of carbon tetrachloride which possesses no bands interfering with the water band. A number of applications have been described recently by Wright (*304*).



Courtesy, W. A. Shurcliff, Calco Chemical Division, American Cyanamid Co.

FIGURE 57. (*Above*) LOG EXTINCTION CURVES FOR CALCOCID VIOLET 4BX. (*Below*) LOG EXTINCTION CURVES ADJUSTED VERTICALLY BY CONCENTRATION FACTORS

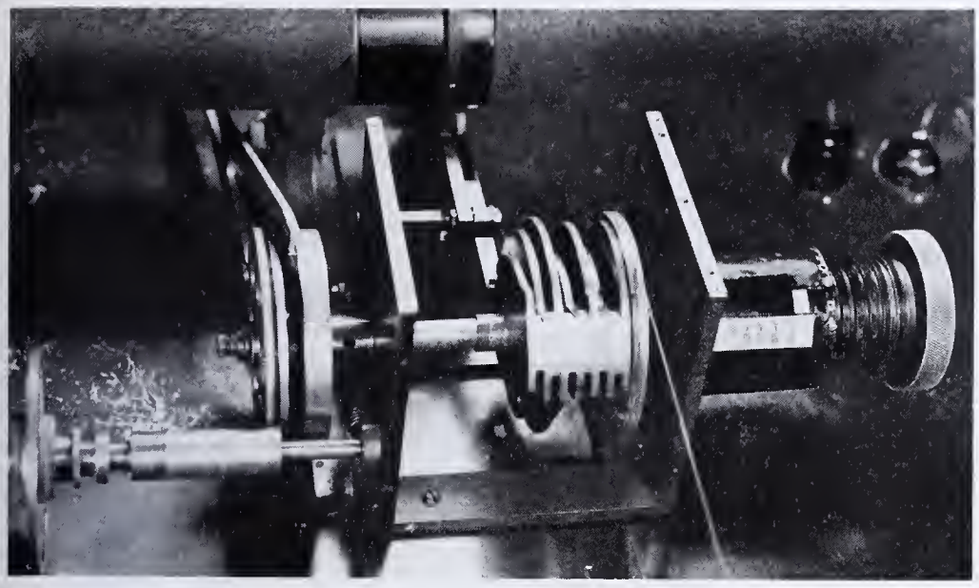


FIGURE 58 (*Above*). SPECTROPHOTOMETER CAM ASSEMBLY

Log log $1/T$ cam on right

Courtesy, W. A. Shurcliff, Calco Chemical Division, American Cyanamid Co.

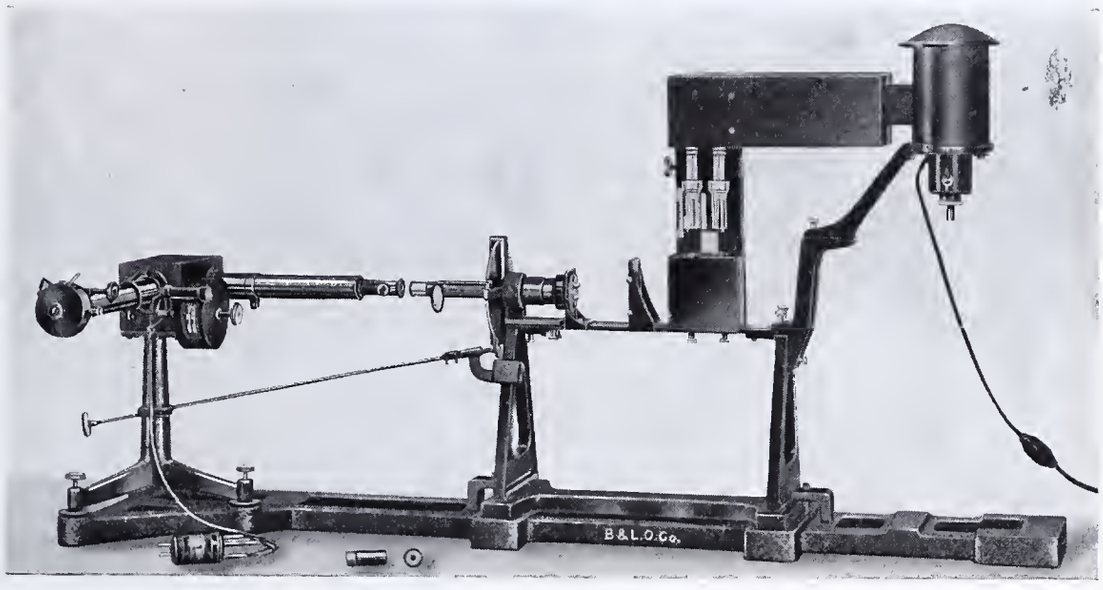


FIGURE 59 (*Right*). POLARIZATION-TYPE SPECTROPHOTOMETER

Courtesy, Bausch & Lomb Optical Co.

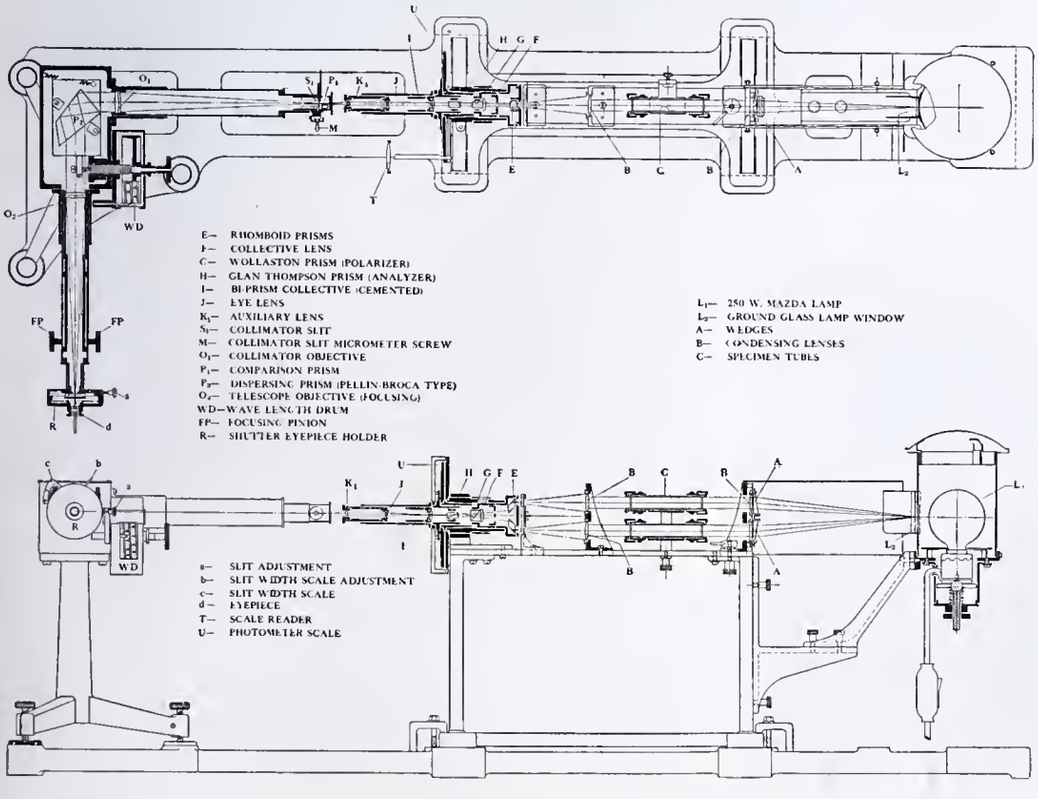


FIGURE 60 (*Below*). POLARIZATION-TYPE SPECTROPHOTOMETER

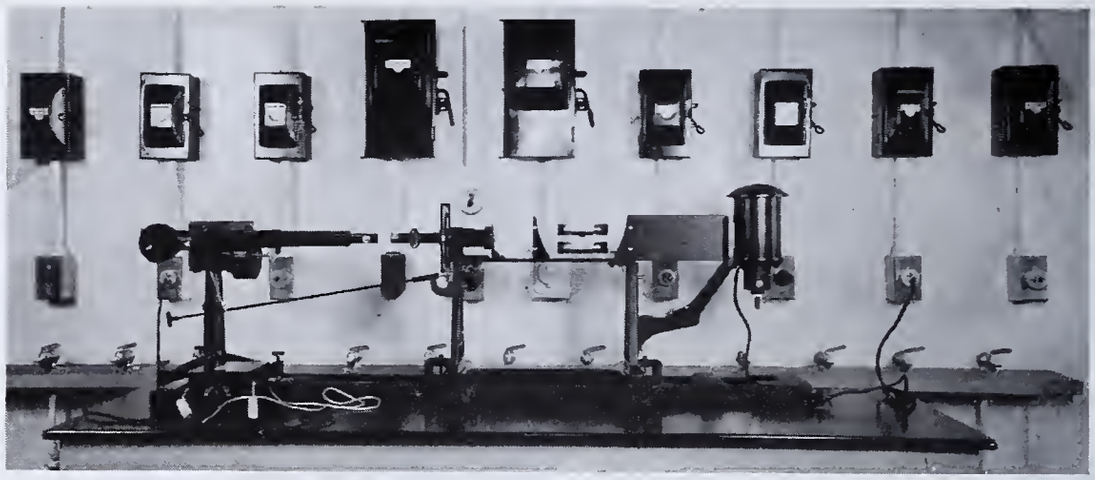
Courtesy, Bausch & Lomb Optical Co.

Raman Spectra

The information afforded by Raman spectra is closely related and complementary to that derived from infrared studies. The Raman effect is based on the observation that the light scattered from molecules which are illuminated with monochromatic light contains frequencies that were not present in the incident radiation. The differences in frequencies correspond to definite energy states in the molecules and are therefore characteristic of the structure of the particular molecule. One great advantage of the Raman technique is that one works in a photographically accessible region and therefore has that advantage over the complicated technique necessary in the infrared. Since the Raman scattering is very feeble, the necessary exposures are rather long.

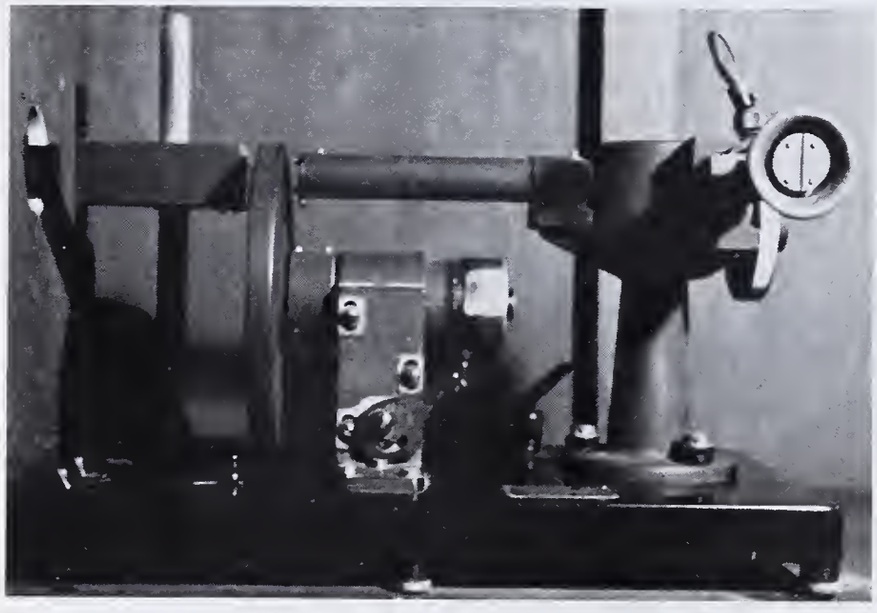
An enormous amount of information has been accumulated in the 13 years since the discovery of the phenomenon and there are several excellent monographs on the subject (*100, 128*).

INSTRUMENTS AND METHODS. The equipment for obtaining Raman spectra is described in great detail in several textbooks. The method consists, in general, of some means for subjecting the substance to intense illumination with



Courtesy, G. I. Lavin, Rockefeller Institute for Medical Research

FIGURE 61. SPECTROPHOTOMETER FOR VISIBLE REGION (BAUSCH & LOMB)



Courtesy, F. W. Zerban, New York Sugar Trade Laboratory

FIGURE 62. KEUFFEL AND ESSER SPECTROPHOTOMETER

Used for color and turbidity determinations by transmittancy in sugar products. Dark room held at constant temperature

monochromatic light and the use of a fast spectrograph for photographing the scattered light with particular precautions for the elimination of stray light reflected from the illuminated vessel. The illumination vessels have taken a great variety of forms (*100*).

A typical vessel consists of a cylindrical tube provided with a plane optically flat window at one end, with the other end of the tube drawn out into a bent horn, which is blackened to minimize reflection. The sample tube is usually surrounded with a cooling jacket and highly selective filters are used to isolate the exciting line from a suitable source, usually a mercury arc. Numerous arrangements have been suggested to provide an efficient "light-furnace", so that the sample may be submitted to the highest possible amount of lateral illumination. The spectrograph is sighted on a portion of the sample in such a manner that the maximum amount of scattered light may be collected, yet avoiding all direct reflection from the source.

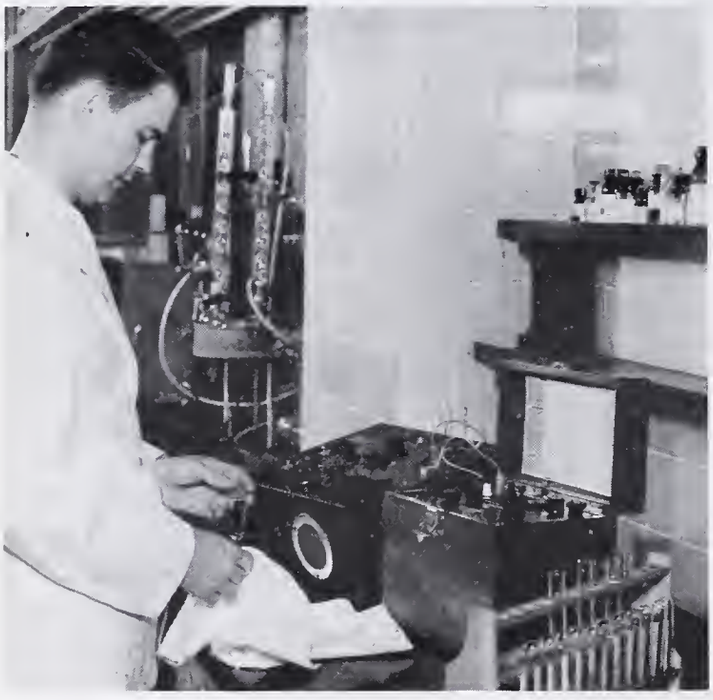


FIGURE 63. DOUBLE MONOCHROMETER SPECTROPHOTOMETER IN CHEMICAL RESEARCH

Courtesy, Coleman Electric Co. and Armour and Co.

APPLICATIONS. The qualitative identification of substances is comparatively simple and amounts to accurate determination of the frequencies of the Raman lines which appear on the spectrogram. The Raman frequencies for an enormous amount of compounds have been recorded (*128*) and reference to these tables permits identification of the substance. In general, the sensitivity of the method is much less than one ordinarily associates with other spectroscopic methods, which is largely due to the feeble intensities characteristic of the Raman effect. In most cases a substance present to the extent of about 1 per cent can be detected, but under

Courtesy, Interchemical Corp.

FIGURE 64. GENERAL ELECTRIC RECORDING SPECTROPHOTOMETER

molecular interaction can arise. Procedures have been developed (*47*, *100*) utilizing a technique similar to the Gerlach principle of homologous pairs of lines and comparing their relative intensities. By calibration with mixtures of known composition very satisfactory results can be obtained with a precision of ±10 per cent without the use of a microphotometer. With the full resources of microphotometry and the usual plate calibration, somewhat more precise evaluation can be made. For further details the reader is referred to Goubeau (*100*), who lists 97 references, most of them concerned with analytical applications. Specific examples are also given by Cringler (*47*) and Hibben (*128*).

X-Rays

The analytical uses of x-rays comprise a very small fraction of all contributions which this subject has made to physical science. Their use in medical radiography, inspection of materials, the elucidation of structure of crystals, etc., is well known and has been set forth in many treatises (*39*, *50*, *122*, *187*, *241*, *254*, *305*), which also discuss analytical uses. Some of the distinctly analytical uses may be listed as shown on the next page.

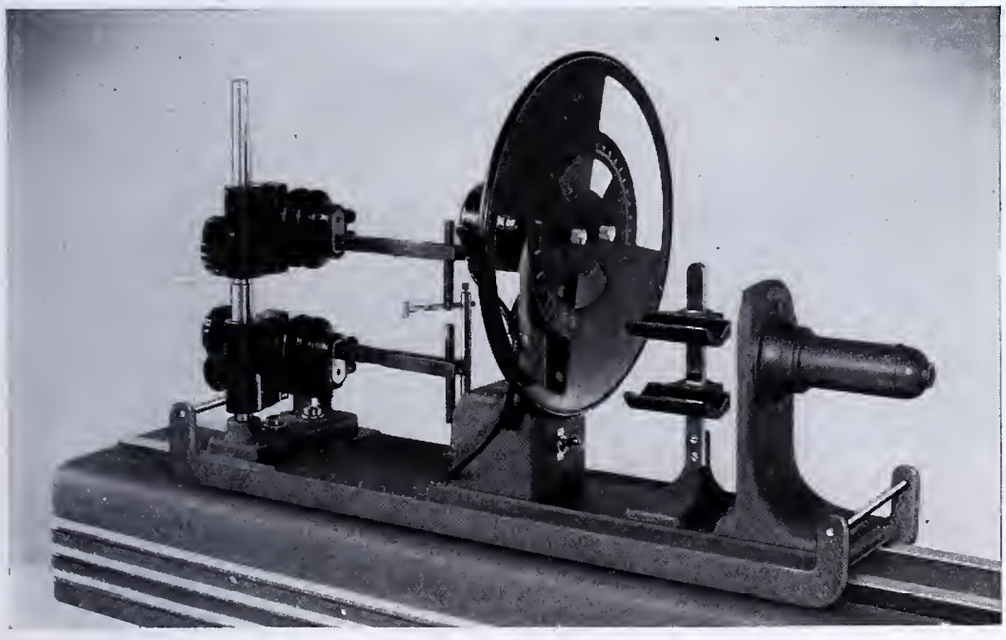


FIGURE 65 (*Left*). ROTATING SECTOR

Courtesy, Bausch & Lomb Optical Co.

FIGURE 66 (*Below*). SPEKKER PHOTOMETER WITH SMALL QUARTZ SPECTROGRAPH

Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.

favorable circumstances to 0.1 per cent. A few examples from Goubeau (*100*) illustrate this point. The detectable limits were found to be 1 to 2 per cent for benzene in dioxane or *vice versa;* 1 per cent for *trans*-crotonaldehyde in *cis*-crotonaldehyde; 0.5 per cent for *p*-xylol in *m*- or *o*-xylol; 0.1 per cent for benzene in carbon tetrachloride.

Quantitative analyses based on the Raman effect are feasible, but these involve all the usual difficulties associated with photographic photometry. Theoretically mixtures of several substances can be analyzed, but the number of Raman lines increases very rapidly with the number of components in the mixture. Certain other complications arise with respect to both the intensity and the frequency, if the system is one in which

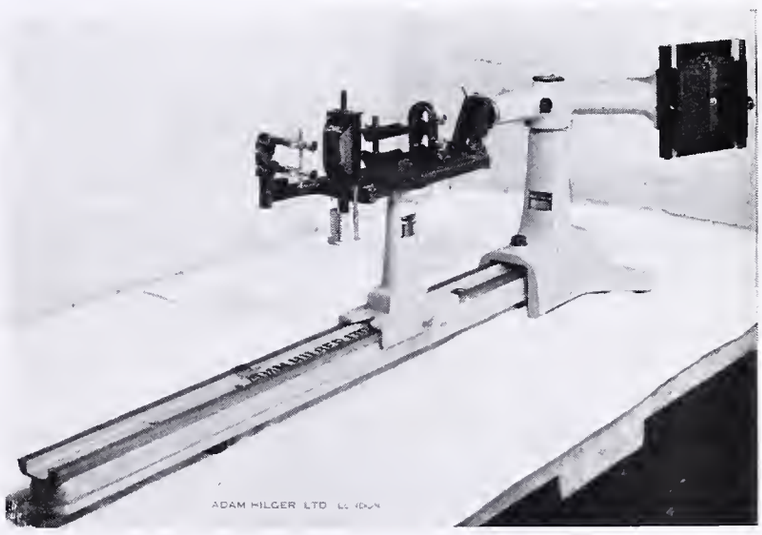
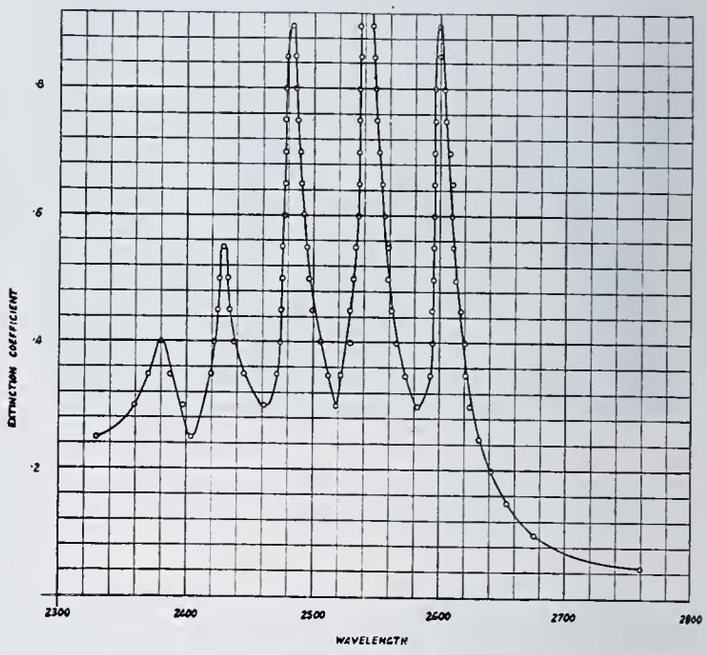


FIGURE 67 (*Left*). ABSORPTION PHOTOGRAPH OF BENZENE (IN HEXANE)

Taken with Spekker ultraviolet photometer and medium all-metal quartz spectrograph. Length of tube 2.0 cm. Minimum exposure 5 seconds

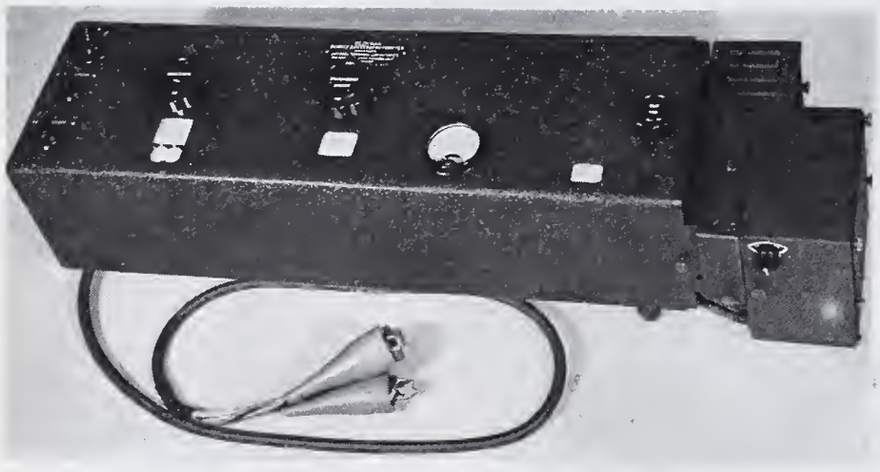
Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.



Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.

FIGURE 68. ABSORPTION CURVE OF BENZENE (IN HEXANE)

Strength of solution, 1 in 3300



Courtesy, National Technical Laboratories

FIGURE 69. BECKMAN PHOTOELECTRIC QUARTZ SPECTROPHOTOMETER

4. Measuring the wave lengths of the characteristic absorption edges when the unknown substances act as the absorbing medium.

5. Determination of the lattice constants of the material, from which the nature of the substance may be inferred, and from the intensity of the lines of the pattern its amount may be inferred.

The possibility of identification presupposes that the lattice constants characteristic of that substance have already been determined—that is, it is impossible to infer the nature of the substance if no values have been recorded for its lattice parameters.

1. Measuring the spectral emission lines (K, L, M, series) in which the unknown substance is the target of an x-ray tube. This method on the basis of Moseley's law has accounted for the original discovery of a number of the elements (Hf, Ma, Re).

2. Measurement of the secondary fluorescence lines emitted by the substance when it is irradiated by x-rays inside the x-ray tube (*14*).

3. The same technique of fluorescence emission with the sample placed outside the x-ray tube, identical with No. 2 but affording somewhat lower intensities.

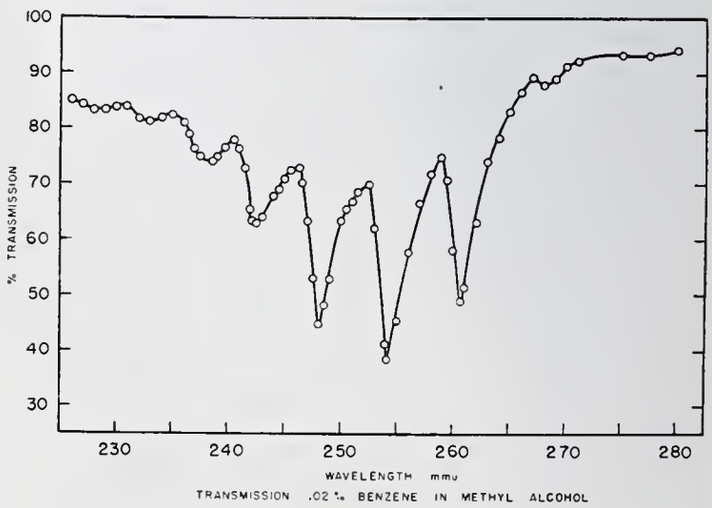


FIGURE 70 (*Right*). ABSORPTION SPECTRUM MEASURED WITH BECKMAN PHOTOELECTRIC QUARTZ SPECTROPHOTOMETER

Courtesy, National Technical Laboratories

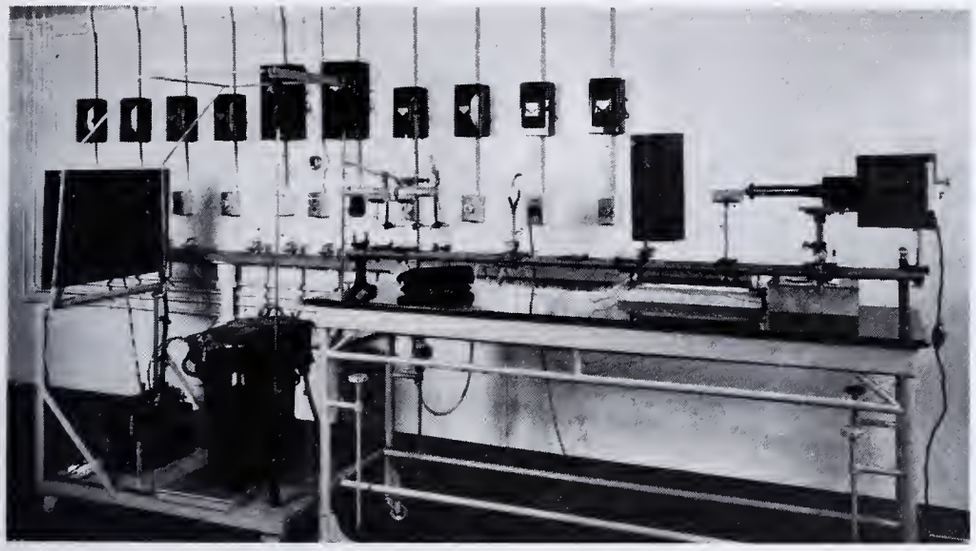


FIGURE 71 (*Left*). INSTALLATION FOR ULTRAVIOLET ABSORPTION SPECTRA

Using quartz hydrogen discharge tube and small Hilger spectrograph

Courtesy, G. I. Lavin, Rockefeller Institute for Medical Research

FIGURE 72 (*Below*). INSTALLATION OF SPEKKER SPECTROPHOTOMETER (HILGER)

Courtesy, G. I. Lavin, Rockefeller Institute for Medical Research

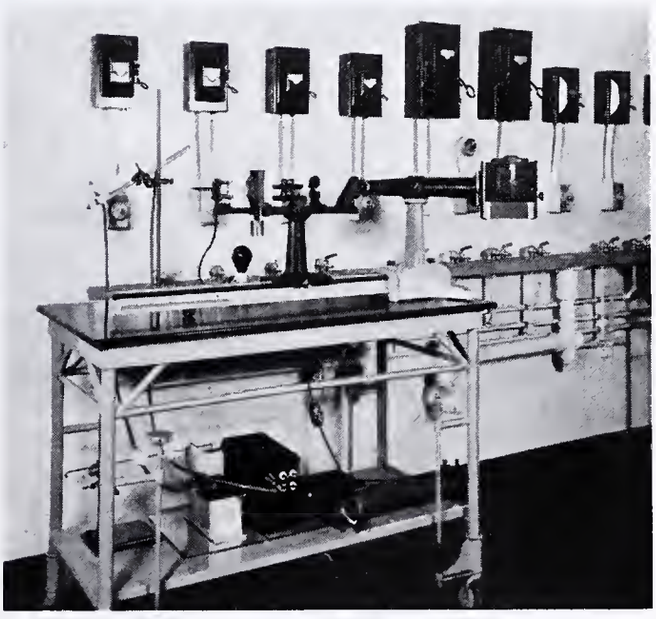
THEORY. The interplanar distance is given by Bragg's law

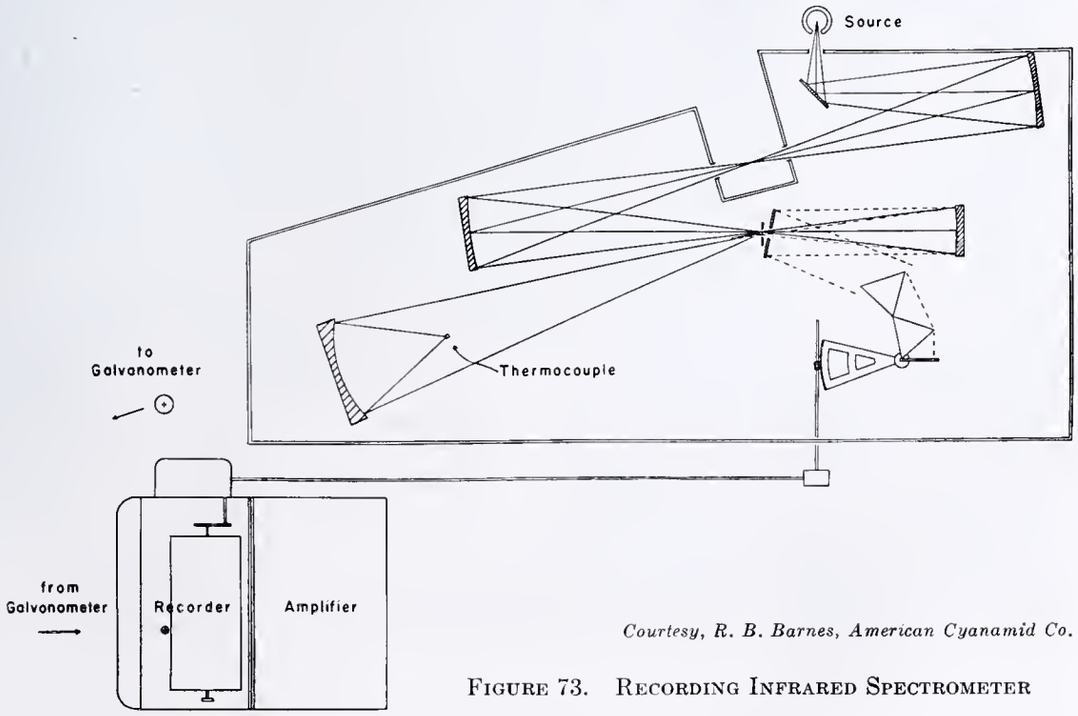
$$n\lambda = 2d \sin \theta$$

This is the fundamental law for the analysis of structure of crystal in substances. Very slight departures from this law are due to refraction, but these corrections are known and have to be applied only for high orders of reflection.

The theory of various space groups and the interpretation for crystals of various classes are the subject of exhaustive treatment in many standard tests.

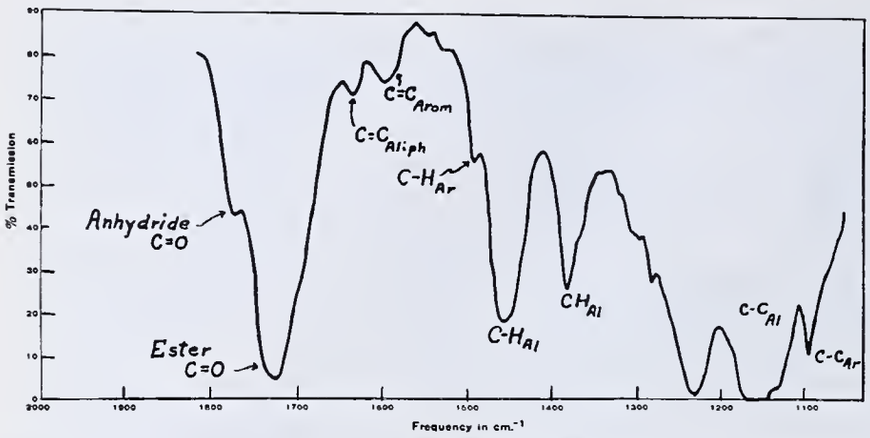
INSTRUMENTS AND METHODS. The variety of instrumental approaches to x-ray analysis is evident in Figures 81 to 91. By exposing a substance to a narrow monochromatic beam of x-rays and recording the diffracted beam on a flat plate behind the sample (Figure 83) the familiar Laue pattern is produced (Figure 85). Interpretation of these patterns is described in detail in all the principal references. In the powder method the camera surrounds the specimen as a concentric cylinder (Figure 86) and diffraction patterns can





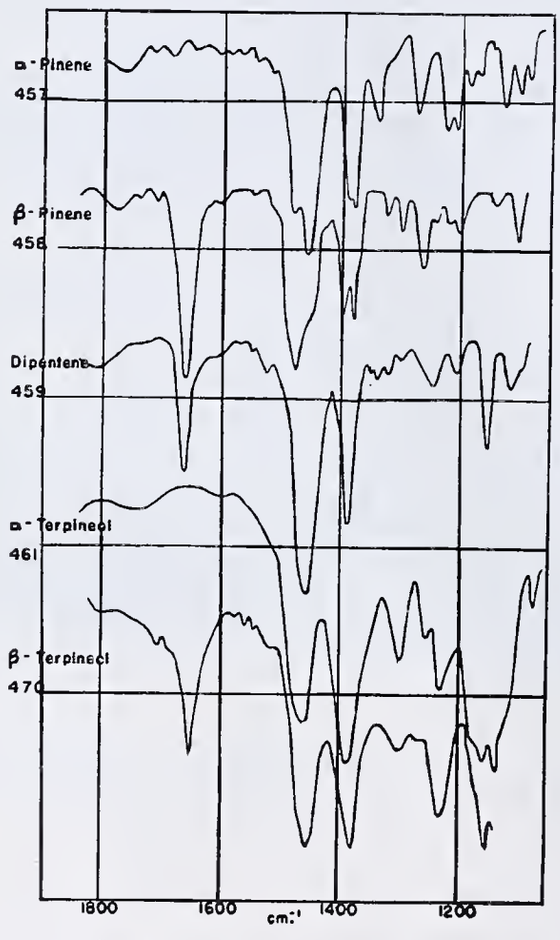
Courtesy, R. B. Barnes, American Cyanamid Co.

FIGURE 73. RECORDING INFRARED SPECTROMETER



Courtesy, R. B. Barnes, American Cyanamid Co.

FIGURE 74. INFRARED ABSORPTION SPECTRUM OF A RESIN, WITH IDENTIFICATION OF BAND HEADS



Courtesy, R. B. Barnes, American Cyanamid Co.

FIGURE 75. INFRARED ABSORPTION SPECTRA

be registered rapidly and in a form which facilitates edge-to-edge comparison with standard patterns. The information obtained by this method involves perhaps the minimum difficulties of interpretation. The back-reflection camera is particularly suited for the precise determination or comparison of lattice parameters.

Most x-ray apparatus intended for general analytical work is provided with means for mounting interchangeable accessories, so that information may be obtained by any of the above-mentioned methods (Figures 92 and 93). Each method has its particular advantages with respect to precision, sensitivity, and ease of interpretation.

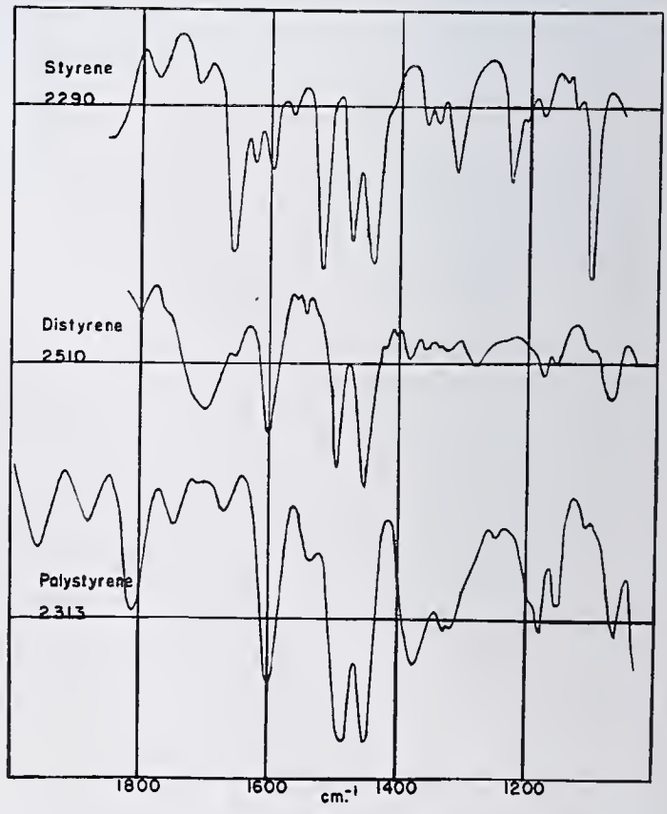
Many accessories are also available for the measurement and rapid evaluation of the patterns (Figure 94). Full details are given by Clark (*39*). No mention is made here of x-ray spectrometers of extraordinarily high precision, such as the double crystal spectrometer. These are available but are intended primarily for fundamental research on lattice constants or conversely for studies on the fine structure of x-ray lines. The analyst is not concerned with their use, although he is always appreciative of the exact information which they can provide.

APPLICATIONS. A list of applications is to be found in monographs and in several interesting papers, such as (*1*).

A recent compilation by Hanawalt (*107*) lists over 1000 substances in terms of the three strongest lines on the diffraction pattern with the respective interplanar distances. The efficiency and convenience of their index are amply demonstrated and it is shown that if the unknown substance is identical with one of the 1000 its presence may be certified in a few minutes by reference to the catalog.

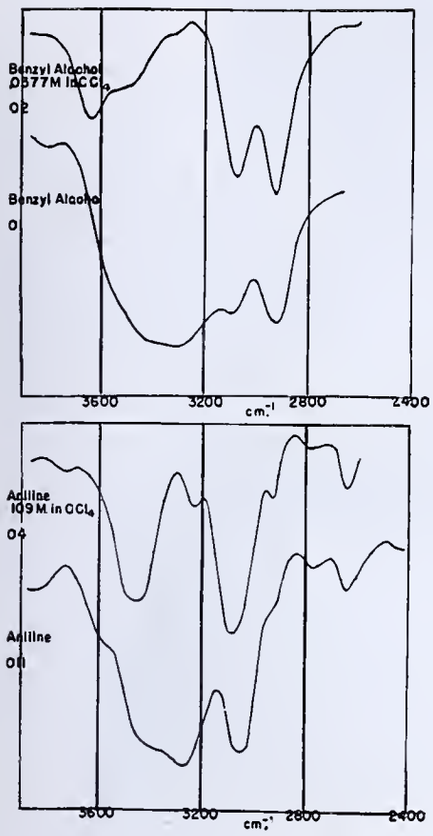
One distinctive feature of the x-ray method, and one which should be kept in mind in comparing it with alternative methods of analysis, is the fact that it identifies compounds. In other words, if one is dealing with a system containing sodium, potassium, chlorine, and bromine, it is possible to tell whether one is dealing with sodium chloride and potassium bromide or sodium bromide and potassium chloride.

The many applications of these methods to analytical problems are the object of an extended review which is now being prepared.



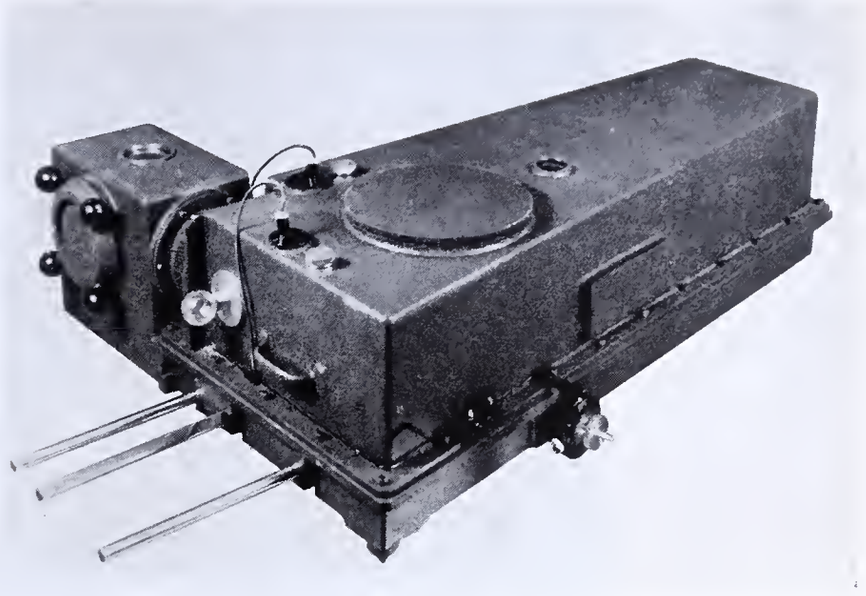
Courtesy, R. B. Barnes, American Cyanamid Co.

FIGURE 76. INFRARED ABSORPTION SPECTRA



Courtesy, R. B. Barnes, American Cyanamid Co.

FIGURE 77. INFRARED ABSORPTION SPECTRA



Courtesy, Gaertner Scientific Corp.

FIGURE 78. LARGE INFRARED SPECTROGRAPH

Electron Diffraction

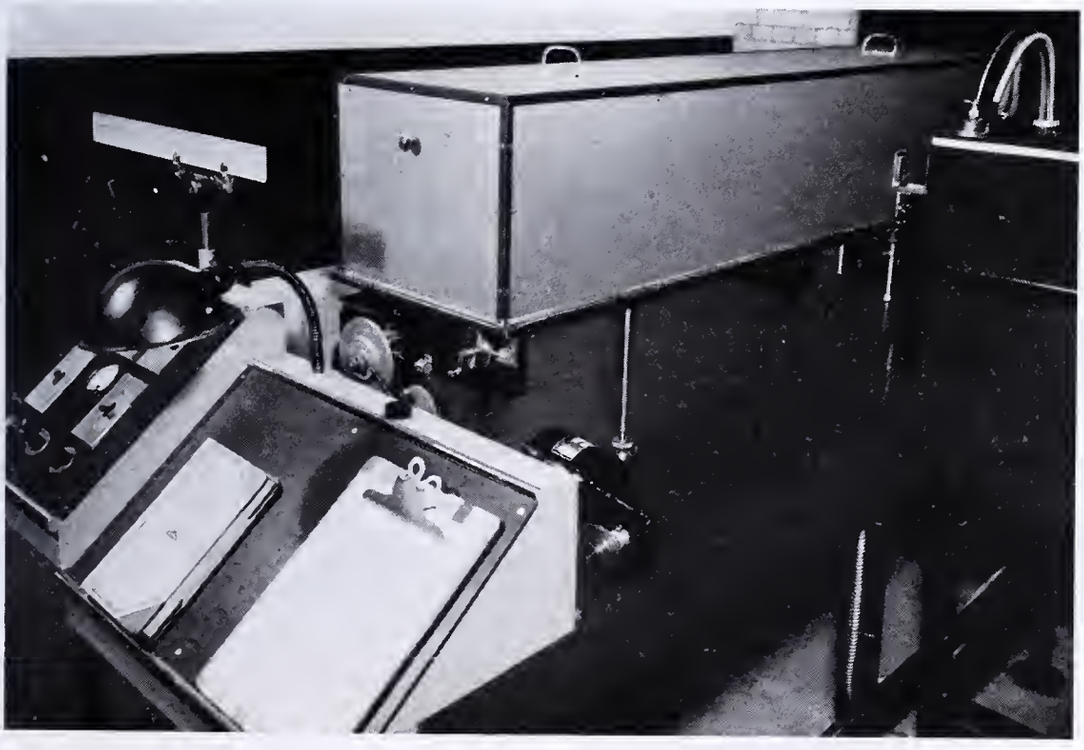
The wave nature of the electron is expressed by the de Broglie equation

$$\lambda = h/mv$$

where λ is the wave length, m and v are the mass and velocity of the electron, respectively, and h is Planck's constant. The numerical value in Ångström units is given by

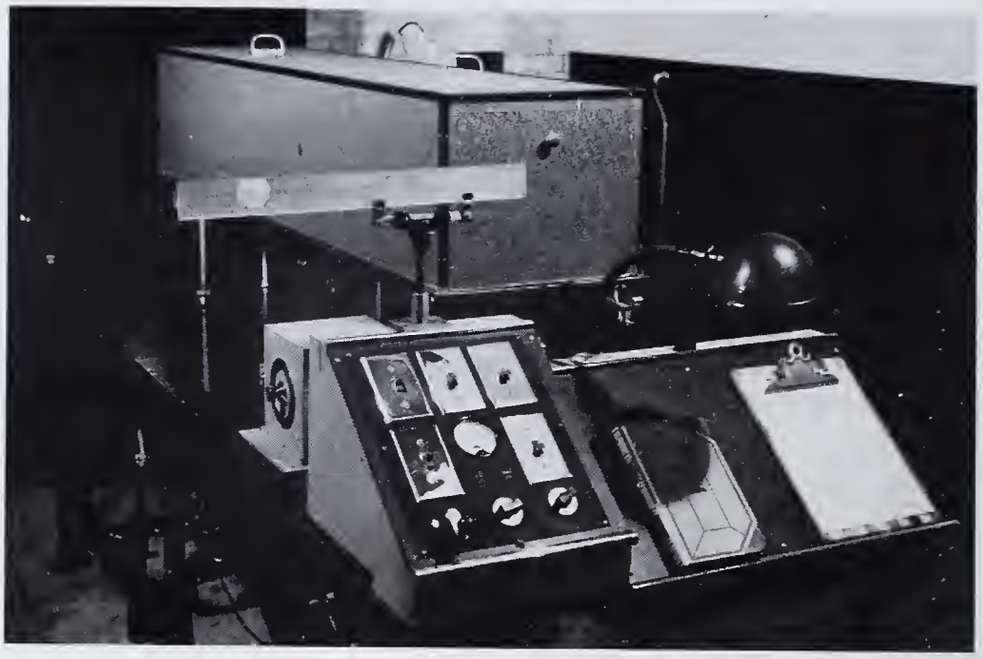
$$\lambda_{\text{Å.}} = \sqrt{\frac{150}{V}}$$

where V is in volts.



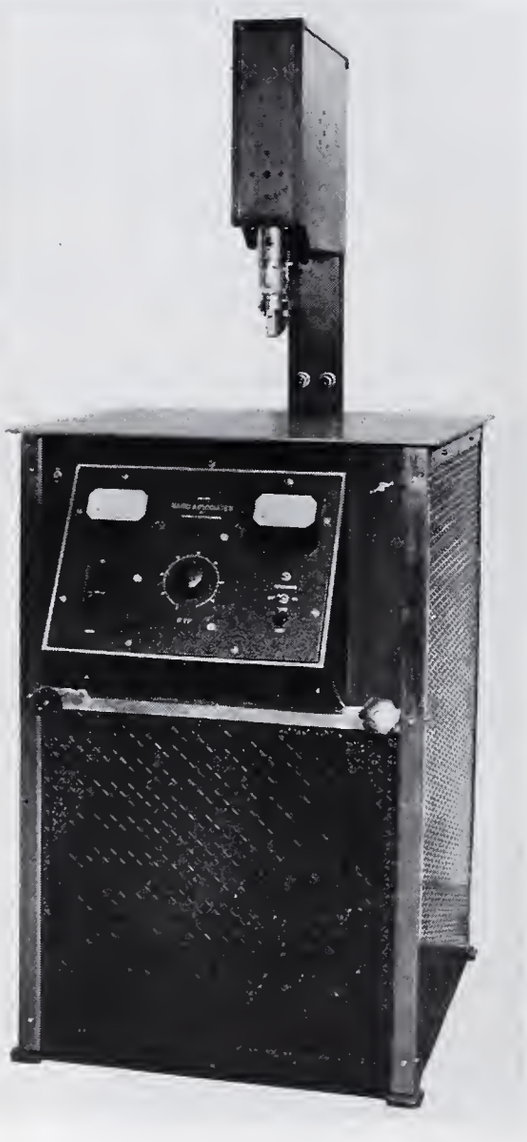
Courtesy, Dow Chemical Co.

FIGURE 79. RECORDING INFRARED SPECTROGRAPH



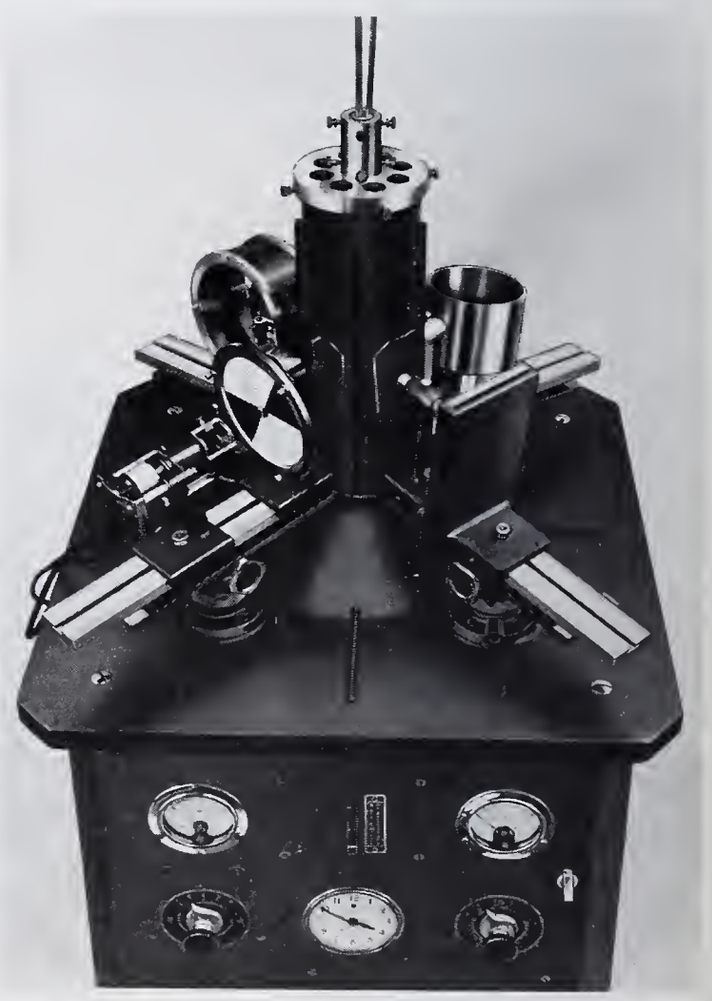
Courtesy, Dow Chemical Co.

FIGURE 80. RECORDING INFRARED SPECTROGRAPH



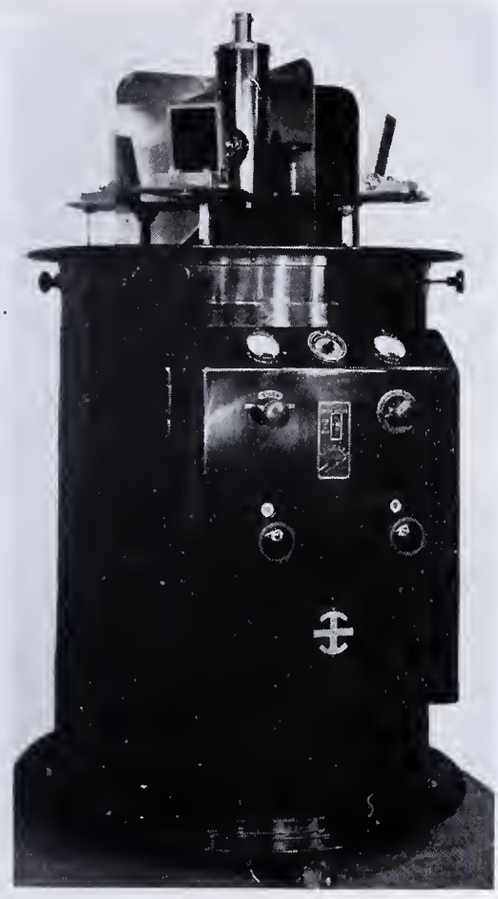
Courtesy, Baird Associates

FIGURE 81. X-RAY DIFFRACTION UNIT FOR RESEARCH



Courtesy, General Electric X-Ray Corp.

FIGURE 82. IN CLOCKWISE ORDER, STARTING AT UPPER LEFT, XRD CYLINDRICAL POWDER CAMERA, BACK-REFLECTION CAMERA, PINHOLE ASSEMBLY AND FLAT CASSETTE, AND UNIVERSAL SPECIMEN MOUNT WITH CYLINDRICAL CASSETTE MOUNTED ON X-RAY DIFFRACTION UNIT



Courtesy, G. L. Clark, University of Illinois

FIGURE 83. MULTIPLE X-RAY DIFFRACTION UNIT (HAYES)

Designed at University of Illinois for photographing four patterns simultaneously

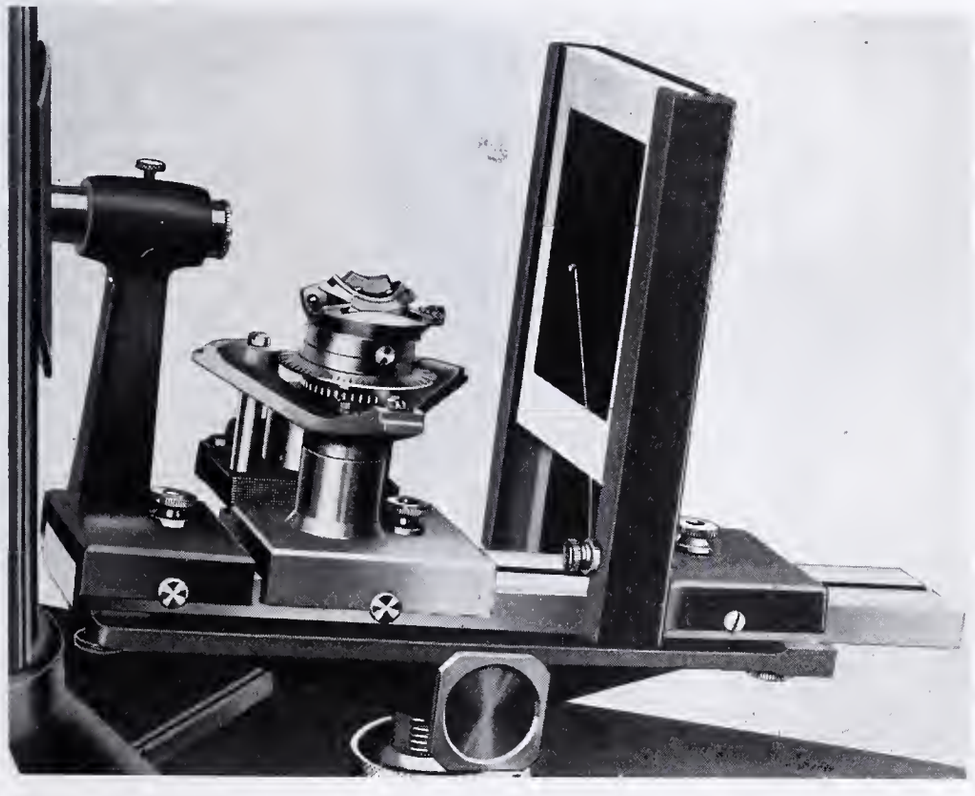
The work of G. P. Thomson, Davisson and Germer, and Rupp has shown that lattice constants may be interpreted from electron diffraction patterns in a fashion analogous to that of x-rays. The technique is primarily suited to films which are thin enough to be penetrated by the electron beams, or to surface films from which they may be reflected. The extension of this method to gases and vapors has been reviewed by Brockway (*29A*).

A typical analytical application is illustrated by the electron-diffraction apparatus of the Dow Chemical Company (Figures 96 and 97), a hot-filament tube operated at $-45{,}000 \pm 3$ volts. The accelerated electrons pass through a 0.2-mm. pinhole in a copper target and are then focused by a magnetic lens before they impinge on the specimen (the adjustable specimen holder accommodates three samples). The pattern made by the diffracted electrons is then recorded on a photographic plate, 18×6 cm. A hexagonal drum in the camera proper carries five plates and a fluorescent screen. The specimen-to-plate distance of the apparatus is 75 cm. Two oil diffusion pumps serve to maintain a vacuum of 10^{-5} mm. of mercury in the entire system. The apparatus was designed and set up by L. K. Frevel and L. Sturkey and has been used primarily in the identification of surface coatings.

Figure 98 reproduces the reflection patterns of magnesium oxide and magnesium hydroxide.

Photometric

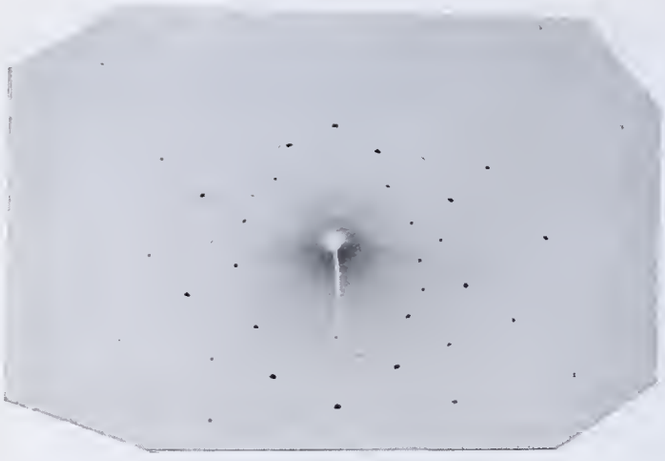
A photometer serves for the comparison of light intensities, which may be from different sources, or from a common source, one portion of the light having been reduced in intensity as a result of absorption or reflection losses. Heterochromatic photometry is of little use in chemical investigations; its use is confined to the measurement and evaluation of illuminants. For all colored substances, it is necessary to restrict the radiation to a narrow region of the spectrum.



Courtesy, General Electric X-Ray Corp.

FIGURE 84. XRD UNIVERSAL SPECIMEN MOUNT WITH PINHOLE ASSEMBLY AND FLAT CASSETTE

For orientation and estimation of degree of internal strain in a single crystal



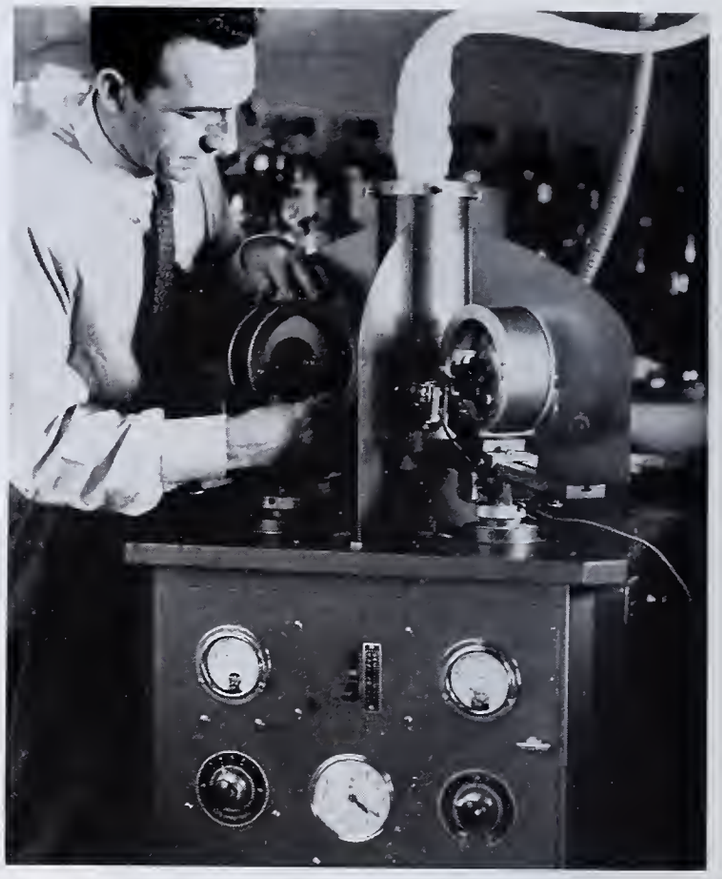
Courtesy, General Electric X-Ray Corp.

FIGURE 85. SLIGHTLY ASYMMETRIC LAUE PATTERN

Obtained from a single sodium chloride crystal, oriented so that x-ray beam is 20 ± 2′ from perpendicular to the [001] direction and 2° 40 ± 2′ from parallel to the [110] direction

This is usually achieved by the use of filters and the instrument is then designated as a filter photometer. In principle it resembles a spectrophotometer, but since the isolation of the spectral region with a filter produces a wider band than dispersion by a monochromator and offers a smaller choice of spectral regions, the instrument is often called an abridged spectrophotometer.

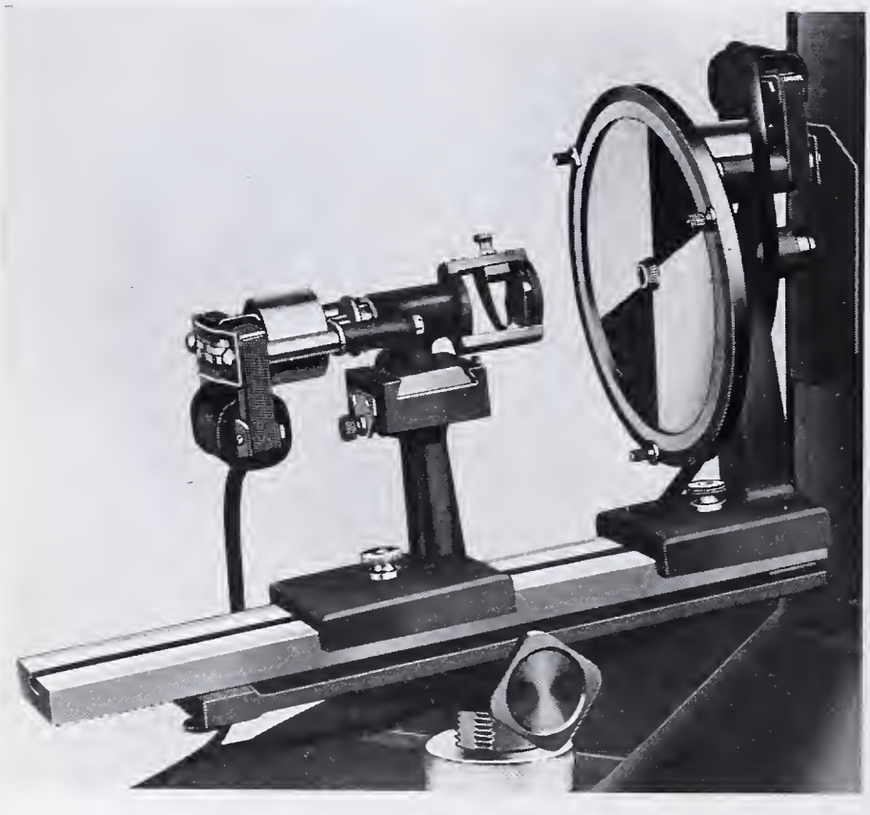
An exception to this point may be made if, for example, a discontinuous source of radiation is used (mercury arc) supplemented by a highly selective filter; it is then possible to isolate a single line and the photometric comparison need not be inferior to that of a spectrophotometer.



Courtesy, Thomas & Hochwalt Laboratories Division, Monsanto Chemical Co.

FIGURE 86. POWDER CAMERA OF X-RAY DIFFRACTION UNIT

Dexter H. Reynolds adjusting powder camera of G-E x-ray diffraction unit. Provision is made for recording diffraction patterns from all types of samples in powder of polycrystalline block form. Diffraction patterns are registered rapidly, and are of a form to facilitate edge-to-edge comparison with standard patterns.



Courtesy, General Electric X-Ray Corp.

FIGURE 87. BACK-REFLECTION CAMERA

XRD camera with 60° window cassette shield in position. Shield permits consecutive registration of three patterns on same film for comparison.

Filter photometers are extremely useful and several commercially available instruments can be had with a large assortment of accessories enabling them to be used for related measurements such as gloss, reflection, albedo, fluorescence, turbidity, and as trichromatic colorimeters, or as comparison microscopes.

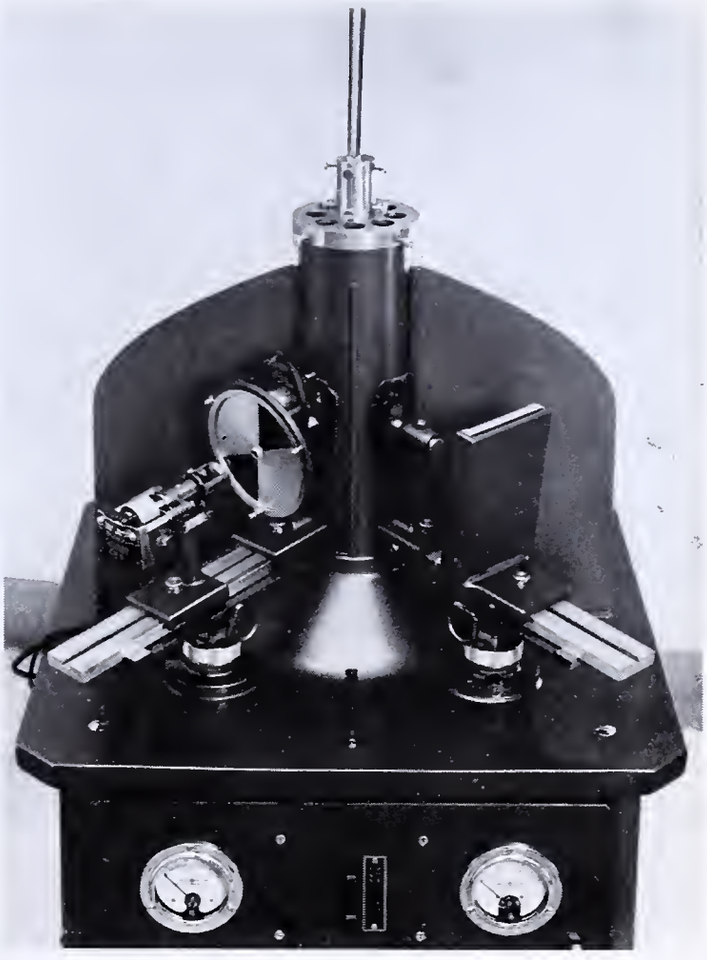
Filter photometers are of two general types—those in which the photometric match is made with the eye and those in which the measurement is effected photoelectrically. The assumption that any photoelectric instrument must be more accurate and reliable than a visual instrument is wholly unwarranted. A very critical and authoritative appraisal of this point has been given by Gibson (*197*). However, the future does seem to lie in the direction of the photoelectric types, for there is no inherent limit in the attainable sensitivity and objectivity of the measurement. Fatigue effects again incline toward the photoelectric type, although squinting at a microammeter needle for protracted periods is not a pleasant pastime.

The relative uses and merits of the filter photometer and spectro-

Courtesy, General Electric X-Ray Corp.

FIGURE 88. BACK-REFLECTION PATTERN OF COLD-ROLLED TEMPERED BRASS STRIP TAKEN WITH SHIELD REMOVED

Variation in intensity around inner diffraction ring permits determination of degree of preferred orientation or texturing induced by cold rolling. Width of diffraction lines is measure of degree of internal strain, a property which determines variations in hardness and strength of polygrained metals.



Courtesy, G. L. Clark, University of Illinois and General Electric X-Ray Corp.

FIGURE 89. XRD UNIT WITH X-RAY TUBE AND CAMERAS

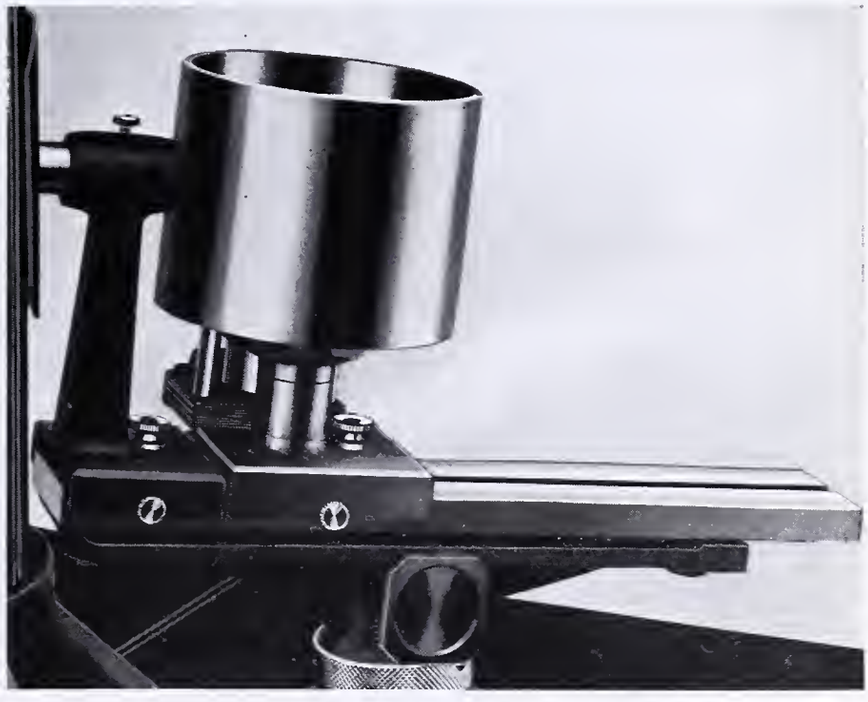
Showing back-reflection camera (*left*) and flat cassette for very accurate measurement of line shifts in solid solutions, etc. (*right*)

photometer must also be approached with common sense, whether one is dealing with the visual or photoelectric type. It is largely the change in extinction with wave length that is important. The photometric matching is equally reliable for the two classes of instrument. In other words, if the substance to be measured possesses very sharp absorption bands, such as hemoglobin or a neodymium salt, a spectrophotometer may be used in any region of the spectrum, but a filter photometer would be restricted to some region not occupied by sharp bands, and therefore probably to one which is very insensitive to small changes in concentration. The error is never unconsciously made (with a visual instrument), however, since the failure of a filter instrument due to such causes is at once apparent—i. e., it is difficult to secure two fields of the same hue.

For those problems in which the absorption bands are broad, and this includes hundreds of important systems, there is little choice between the two, and the less expensive instrument is to be preferred.

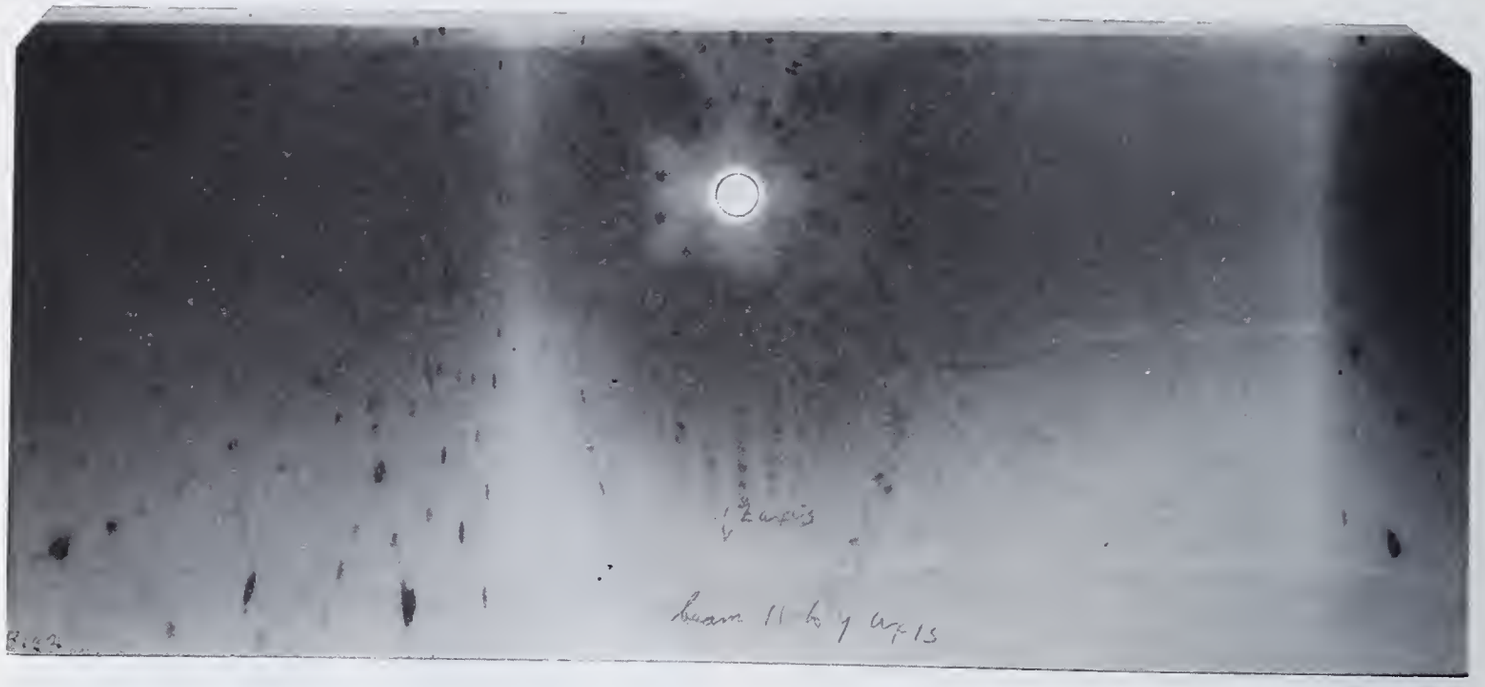
In the measurement of mixtures, the filter photometer is likely to impose empirical calibration, whereas the spectrophotometer can be depended upon to yield additive extinction values. There are other considerations applying to mixtures—i. e., the relative values of the separate extinction coefficients which limit the accuracy (*114*).

THEORY. A filter photometer consists of a light source, an appropriate optical system for producing a parallel beam of light, a container for the light-absorbing sample, or means for reflection from the sample, and means for measuring the



Courtesy, General Electric X-Ray Corp.

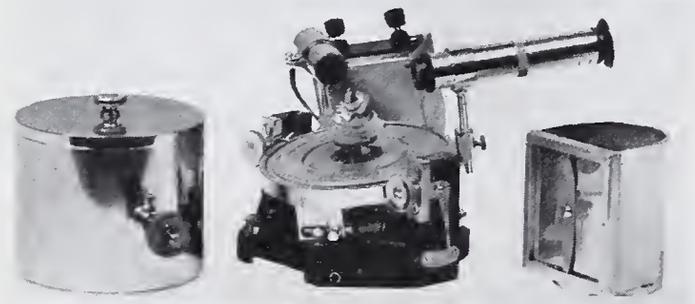
FIGURE 90. UNIVERSAL SPECIMEN MOUNT IN USE WITH CYLINDRICAL CASSETTE



Courtesy, General Electric X-Ray Corp.

FIGURE 91. PATTERN OBTAINED IN CYLINDRICAL CASSETTE MOUNTED ON UNIVERSAL SPECIMEN MOUNT

Records pattern of a stationary quartz oscillator orientated so that x-ray beam coincides approximately with electric axis of crystal and central axis of cassette coincides with optical axis of cassette. Faces of oscillator contain optical axis and make acute angle with electric axis. Characteristic piezoelectric frequency of an oscillator may be predetermined by specifying relation between its physical dimensions and crystallographic axes. Piezoelectric properties are constant over a longer life if this relation meets exact specifications. Routine determination of orientation of crystallographic axes in rough quartz crystals by x-ray diffraction techniques is standard practice with large manufacturers of quartz oscillators.



Courtesy, G. L. Clark, University of Illinois

FIGURE 92. UNIVERSAL CAMERA FOR X-RAY DIFFRACTION (HAYES)

Designed at University of Illinois. *Left*, powder and rotation camera. *Center*, base of camera and goniometer mounting for single crystal (Laue or rotation method). *Right*, mounting of flat film for Laue or fiber pattern.

amount of light which has been absorbed. An approximation to monochromaticity is obtained by suitable filters, placed either before or after the sample. The measurement may be made directly (in some photoelectric instruments) or by comparison with a reference beam (visual and some photoelectric instruments).

Visual Instruments. Two beams of light from a common source, one passing through or reflected from the sample, the other passing through some device which reduces its intensity in measurable amount, are brought together as adjacent fields and are compared with an eyepiece. Three methods for weakening the comparison beam are commonly employed:

1. Aperture type in which a slit or diaphragm is opened or closed. The intensity is a function of the aperture and in the case of a square aperture moving along a diagonal (Zeiss) follows a square law.

$$I = k\theta^2$$

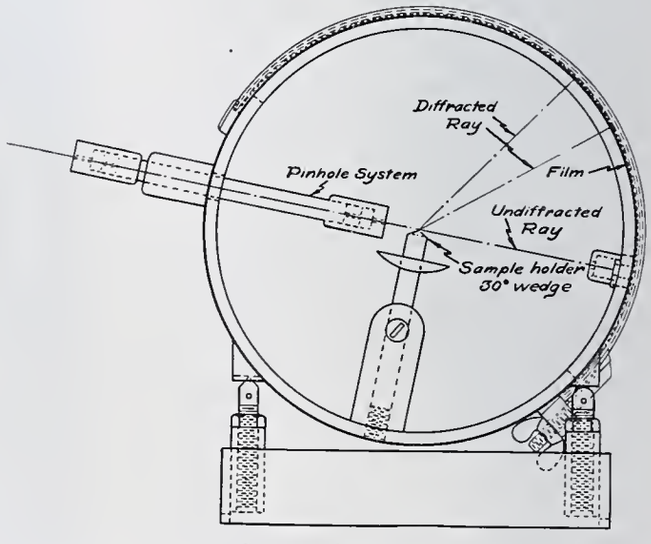
where θ is the angular motion of the feed screw

2. Polarization type in which the comparison beam is rendered plane-polarized and reduced in intensity by a rotatable analyzer. The intensity variation follows the law

$$I = I_0 \cos^2 \theta$$

Tables are usually provided with this class of instrument relating the observed angles to the corresponding transmission, density, or extinction values.

3. Neutral wedge type in which the comparison beam passes through a light-absorbing wedge. The wedge may be moved



Courtesy, G. L. Clark, University of Illinois

FIGURE 93. UNIVERSITY OF ILLINOIS CAMERA FOR POWDER PATTERNS

May be used with appropriate modifications for vacuum and gaseous atmospheres and at high and low temperatures.

Courtesy, General Electric X-Ray Corp.

FIGURE 94. FILM SCALE

XRD film scale, holding two superimposed powder camera patterns in position for measuring angles between corresponding diffraction maxima. Parameters characteristic of crystal structure of unknown samples may be read from standard tables and compared with parameters of known pure substances for absolute qualitative analysis for solid phase constituents. Rough quantitative estimation may be made from relative intensities of diffraction maxima in many cases.

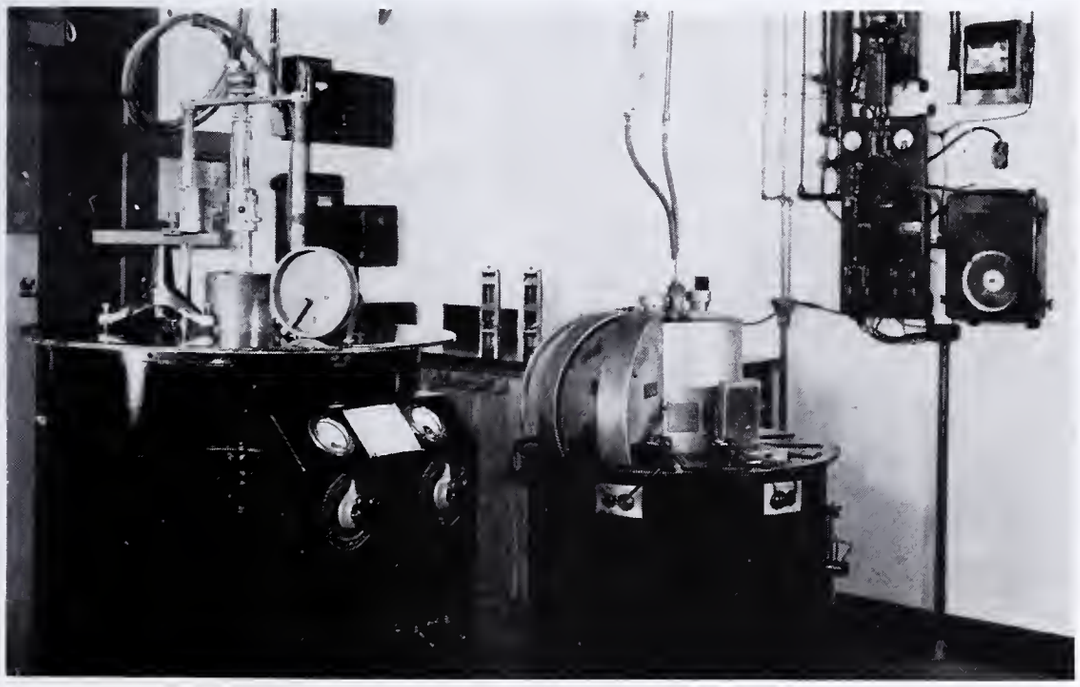
along its length, thus introducing a thinner or thicker portion. To avoid gradients in the effective portion a compensating wedge is often introduced. This is an identical wedge, turned in the opposite direction; consequently the net cross section exposed to the beam is an optically uniform rectangle. Since the intensity varies logarithmically with thickness (Lambert's law), the motion of the wedge is linear in density or extinction.

$$I\alpha - \log T\alpha d\alpha E$$

Photoelectric Instruments. There are many types and variations (*183, 213, 310*), most of which have been classified by the writer (*196*). The photocells may be of the barrier layer, emissive, or multiplier type (*197*). There are single-cell photometers with a choice of electrical methods for measuring the current—i. e., direct deflection, and potentiometric compensation of both linear and logarithmic nature (*196*). Double-cell arrangements (*299*) consist of two photocells illuminated from a common source, with the sample placed in one of the beams. Optical compensation is effected in the other beam, or the two photocurrents may be measured electrically by (*a*) opposed flow through a common meter or galvanometer, or (*b*) potentiometric or shunt cancellation. Some of these circuits compensate for source fluctuations and others do not (*196*).

Calibration and Computation of Results. Many instruments carry scales which indicate directly the measured quantity as well as derived quantities which are more directly applicable. Thus a photometer scale may read per cent transmission, density, or extinction. In others, tables are provided to translate the instrumental indication into the required information. It seems that more care, ingenuity, and real instrumentation have been practiced in the older visual instruments than in the newer photoelectric devices. Density or extinction indications are to be regarded as the ultimate goal in all photometers intended for photometric analysis.

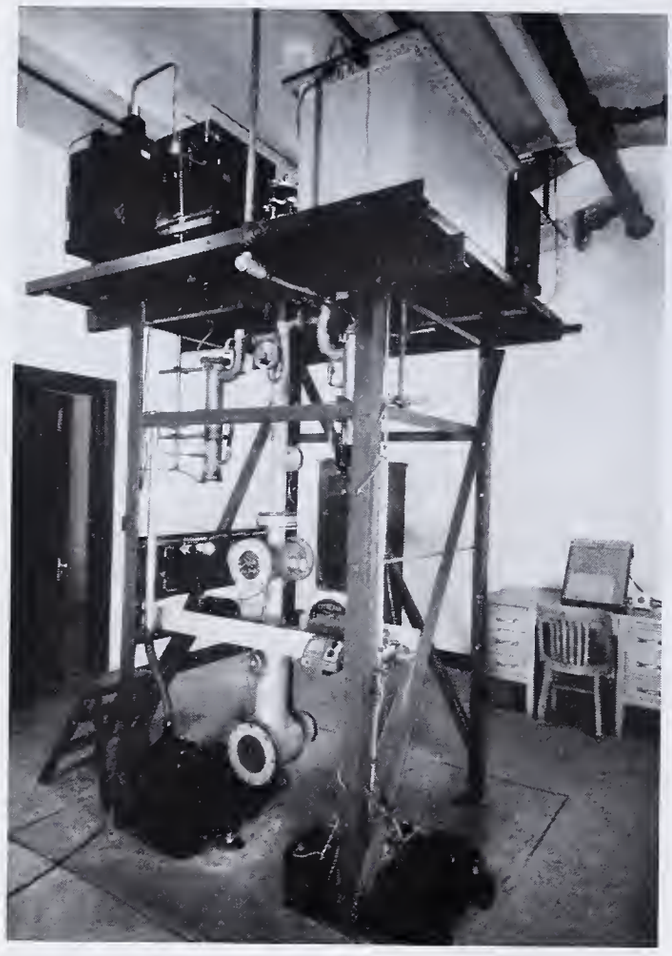
For instruments which indicate the extinction directly, the concentration is directly proportional to the reading, provided Beer's law applies. The present need for empirical calibration with known solutions in so many instances is partly due to lack of good instrumentation and also to our



Courtesy, Dow Chemical Co.

FIGURE 95. X-RAY DIFFRACTION UNITS

Unit at right is equipped with a Mo target tube and is used mainly for routine chemical analysis by powder diffraction method. Orientation studies on metals and other substances are also made with this unit. Unit at left is equipped with a Cu target tube and is used for precision measurements of lattice parameters and for x-ray studies of polymeric substances.



Courtesy, Dow Chemical Co.

FIGURE 96. ELECTRON DIFFRACTION APPARATUS

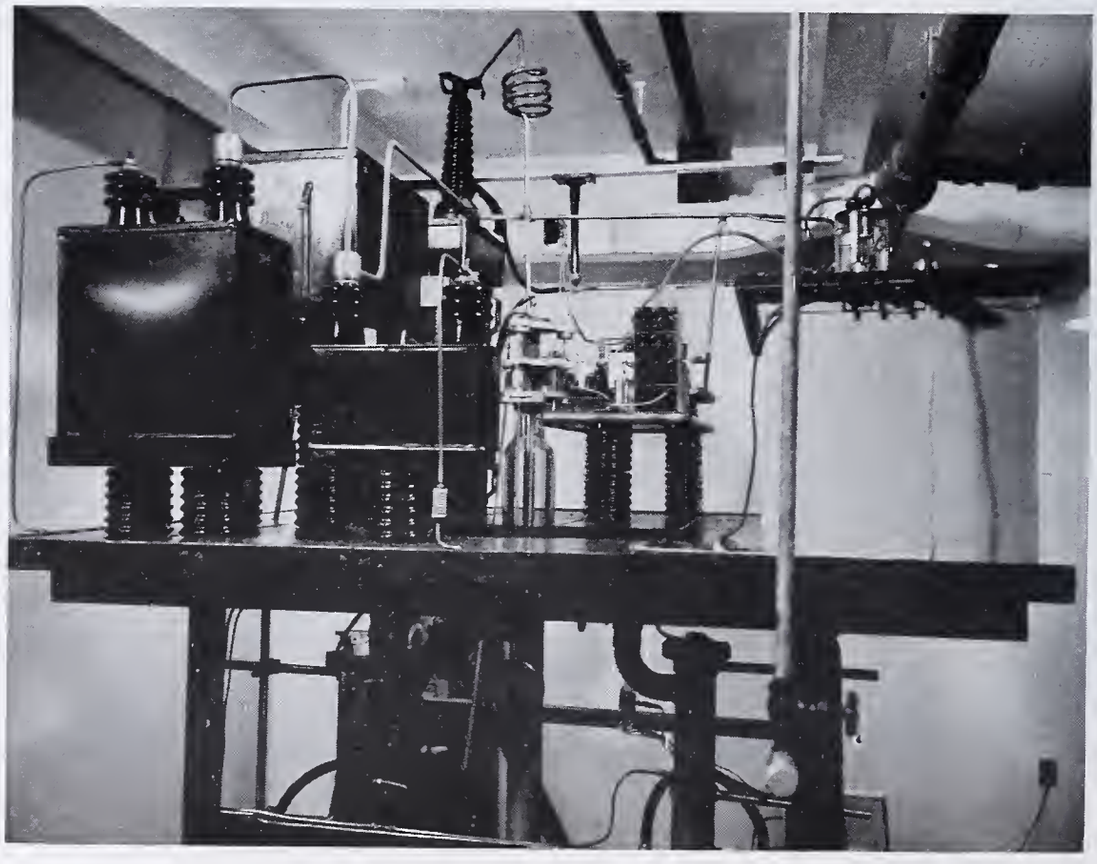
limited and still unsatisfactory knowledge of "colorimetric" chemical reactions.

An important question arises in connection with all filter photometers: To what extent can measurements reported by one investigator be duplicated by another, using a different instrument? No question of this sort arises in the use of a spectrophotometer. In some filter photometers, the photometric system is sufficiently fundamental in design and the filters are held in such rigid absolute specifications that it is possible to publish "effective extinction coefficients" for different substances with reasonable certainty that others can duplicate the results.

INSTRUMENTS. Of the many instruments available for this work we shall discuss a few which are representative of a given type or class. Some are unique in their class, others have many successful competitors.

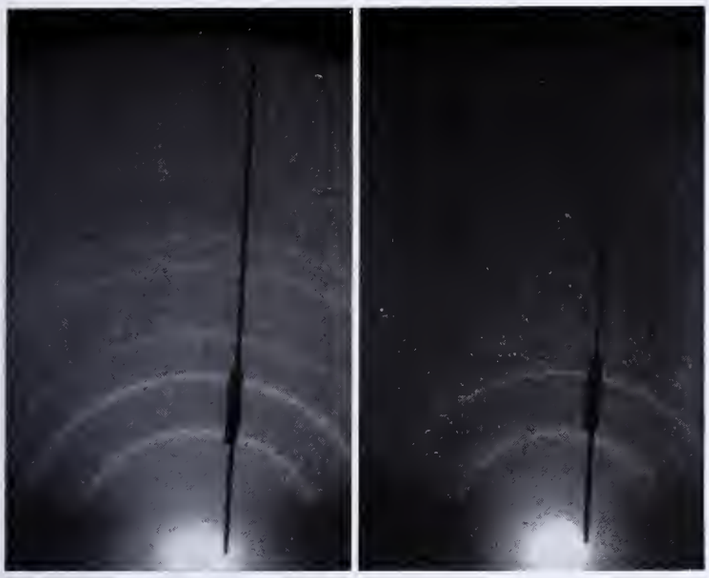
Visual Instruments. Aperture Type. The Zeiss Pulfrich photometer, shown in a general view as Figure 99 and in schematic sectional diagram as Figure 100, is a fine example of this class.

The photometer lamp contains a source, two mirrors, and two adjustable sleeves carrying focusing lenses and ground-glass diffusing screens. The photometer head carries demountable cell holders and two variable apertures which are opened or closed by rotating large measuring drums. The separate beams pass through total-reflection prisms to a biprism and the circular bipartite field is viewed with an eyepiece. A revolving filter holder permits the selection of any one of a dozen highly selective filters, which occupy a position between the biprism and the ocular. The holder normally accommodates nine filters at a time, but special filters are easily snapped in or out of the holder. The filters are extremely selective, almost opaque to normal daylight, and the spectral centers of gravity are more or less uniformly distributed throughout the visible spectrum. Complete specifications of the filters are provided by the manufacturer with respect to wave length at maximum transmission, effective center of gravity, width of band at half and



Courtesy, Dow Chemical Co.

FIGURE 97. ELECTRON DIFFRACTION APPARATUS



Courtesy, Dow Chemical Co.

Magnesium Oxide Magnesium Hydroxide

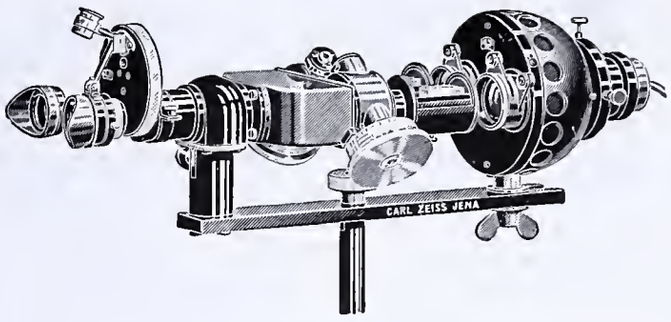
FIGURE 98. REFLECTION PATTERNS OF MAGNESIUM OXIDE AND MAGNESIUM HYDROXIDE OBTAINED WITH ELECTRON DIFFRACTION APPARATUS

tenth of maximum transmission, and transmission at the center of gravity. For example, the S61 filter (yellow) has a band width of 24 mμ at half value and 66 mμ at tenth value, a maximum transmission of 620 mμ, a center of gravity at 619 mμ, and a transmission of 0.080 per cent at this wave length. Special filters are available for use with a mercury arc source to isolate the blue line 436 mμ, the green line 546 mμ, and the yellow pair 577–579 mμ. The aperture drums are both calibrated, and read in per cent transmission and extinction. A very large assortment of accessories can be had to adapt the instrument to any type of photometric measurement. Another very convenient feature, as pointed out by Ashley (*8*) who has made extensive use of this equipment (*7*), is the ease with which absorption cells or tubes varying from a millimeter or less to 50 or more centimeters in length can be accommodated.

This instrument is worthy of the name of Pulfrich, who has contributed so much to the science of optics. Its excellence and utility are best attested by the monographs and voluminous literature devoted to its uses and applications (*114*, *285*, *285A*). Complete accessories are obtainable.

Polarization Type. An example of this class of photometer is shown in Figure 101 and in schematic view in Figure 102. This is another high-class optical instrument of perfectly general utility, which with suitable accessories can be used for every conceivable photometric problem.

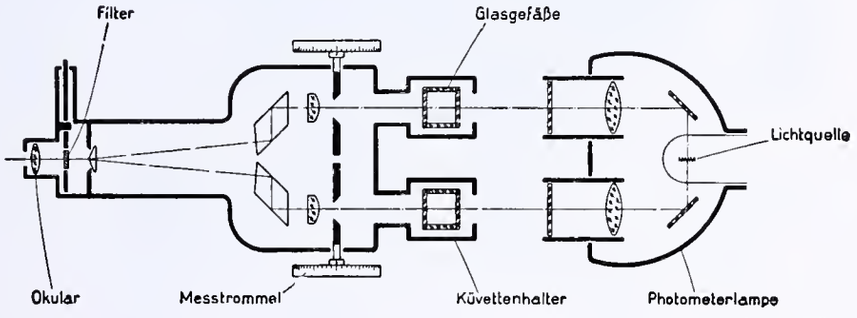
As shown in Figure 102 it is set up for the photometry of colored liquids or solutions. Light entering from the right traverses two total-reflecting prisms and rises through the adjustable absorption cell, after which it passes through a Brodhun photometer cube, through another total-reflecting prism, and out through the eyepiece and color filter. A portion of the original light beam is reflected upward on the right side of the instrument, where it is reflected from an adjustable mirror and enters the polarizing head. The second Nicol prism can be rotated to decrease the intensity and a scale indicates the angle of rotation.



Courtesy, Carl Zeiss, Inc.

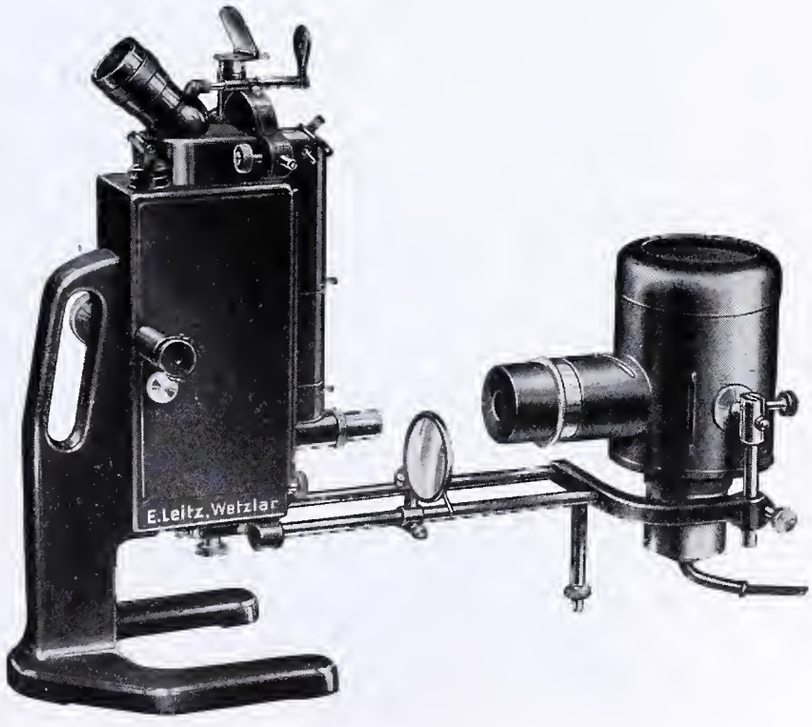
FIGURE 99. ZEISS PULFRICH PHOTOMETER

The emergent beam strikes the Brodhun photometer cube, from whence it passes through the filter and eyepiece. The photometric field appears as two semicircular patches which, upon rotation of the Nicol, can be made to merge into a single uniformly illuminated field. The computations are in terms of the general law for polarization photometers, but to save the observer's time, tables are provided relating extinction to angle of rotation of the analyzing Nicol prism. This versatile instrument has been applied to a great variety of problems (Figure 103).



Courtesy, Carl Zeiss, Inc.

FIGURE 100. SCHEMATIC SECTIONAL DIAGRAM OF ZEISS PULFRICH PHOTOMETER



Courtesy, E. Leitz, Inc.

FIGURE 101. POLARIZATION-TYPE PHOTOMETER

Wedge Type. An example of this class is shown in Figures 104, 105, and 106. This instrument has enjoyed widespread adoption and indicates extinction values directly in a range of 0 to 2.0 (100 to 1 per cent transmission), and hence for all systems obeying Beer's law the scale readings are directly proportional to concentration. The principle is almost self-evident from the detailed illustrations.

The second condenser lens bears a lightly frosted surface which furnishes intensely and uniformly illuminated patches of light. Two wedges are provided, a small compensating wedge in the path of the absorbing system and the larger measuring wedge (100 mm.). The two beams are combined as shown and pass into the eyepiece and color filter. A large selection of highly selective filters is offered, including monochromats for the blue, green, and yellow lines of the mercury arc. This company's excellent series of precision absorption cells are used with the instrument (*197*, Figure 71). The neutral wedge is practically nonselective, showing no more than 6 per cent decrease in transmission at short wave lengths and 3 per cent at long wave length. For studies at any one wave length this is of little consequence. A related instrument of the photoelectric type is discussed later.

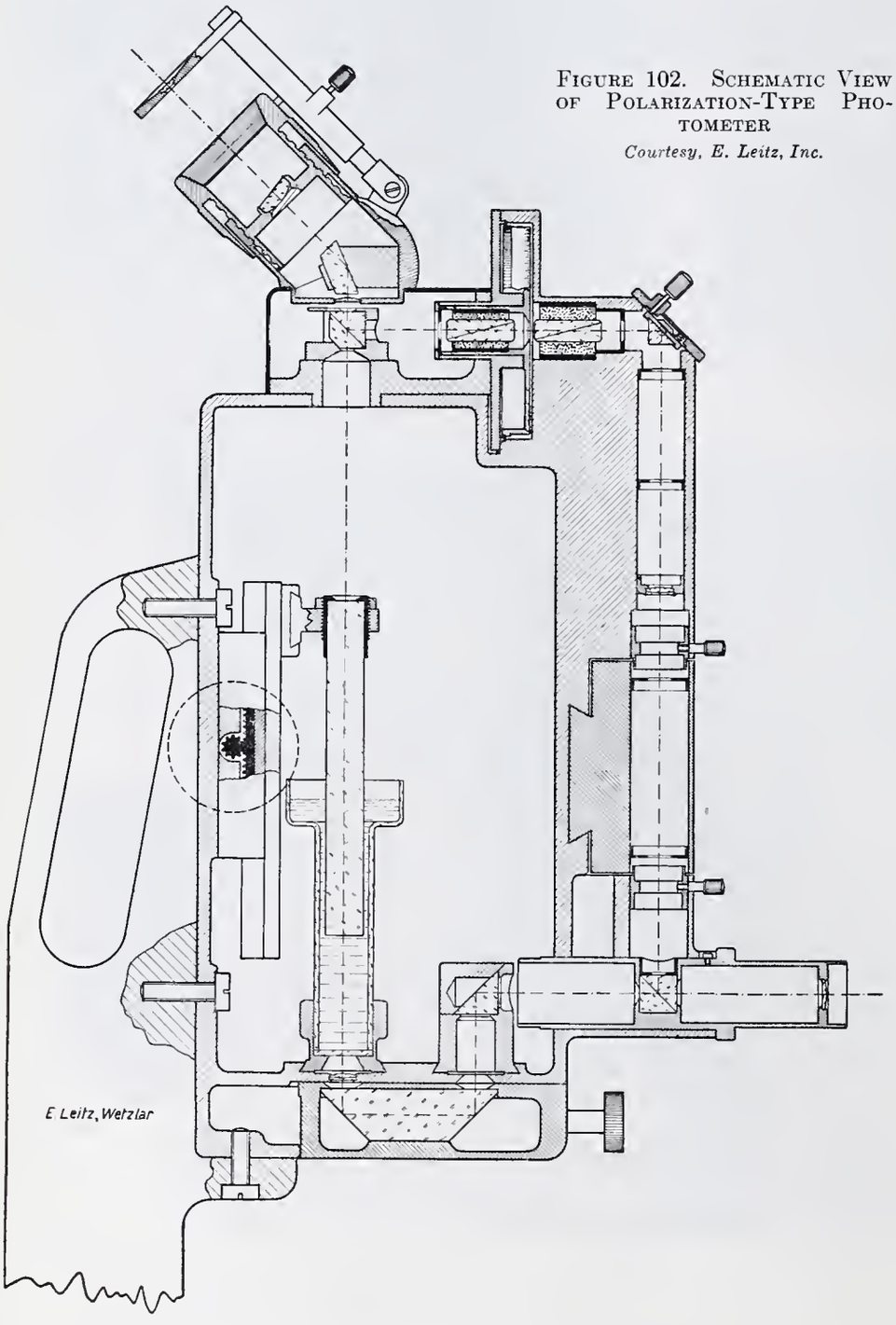


FIGURE 102. SCHEMATIC VIEW OF POLARIZATION-TYPE PHOTOMETER
Courtesy, E. Leitz, Inc.

There is little to be said in appraising the future of visual photometers. To many, their days seem to be numbered in view of photoelectric developments, but the best resources of the last half century's optical theory and practice have gone into their design and they are real instruments in a sense achieved by too few of their photoelectric successors. There is a degree of versatility, constancy, reliability, and ease with which they can be put into service at a moment's notice which is not to be ignored. As instruments they have reached the upper limit—i. e., that set by the visual acuity of the observer.

Photoelectric Photometers. A classification of these methods has been mentioned (*196*) and it may serve our purpose here to illustrate a few type examples.

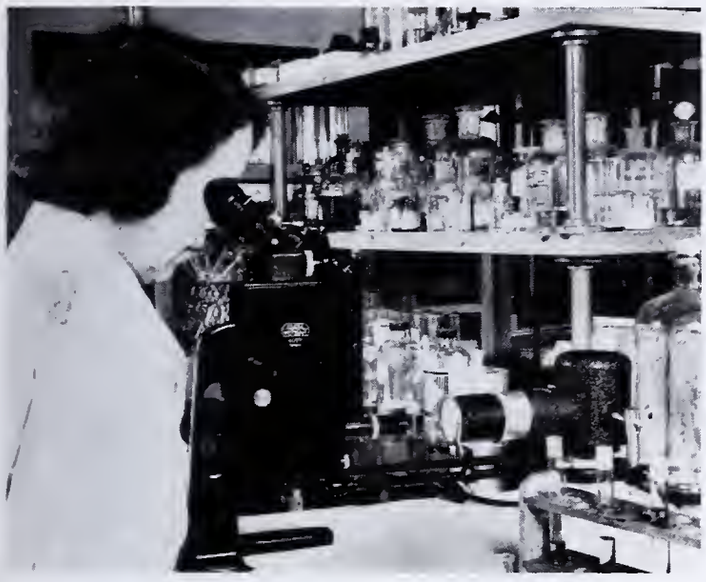
Single-Cell Barrier-Layer Type. In this class, a single photocell of the self-generating type is used to measure the intensity of a semimonochromatic beam of light. Measurements are made alternately through solution and solvent and the ratio of the photocurrents indicates the transmission. Various electrical schemes other than a direct measurement of the current with a microammeter or galvanometer are used (*196*). The method requires constancy of the light source, at least during the interval in which the two readings are made. A well-known and widely used instrument of this type is shown in Figures 107 and 108.

The light source is fed from a constant-current transformer and the photocurrent is read with a Weston microammeter. The absorption cells move in a carriage in a direction perpendicular to the optic axis. A large reading glass inserted in the top of the case facilitates reading the meter. Suitable light filters are supplied, according to the location of the absorbing bands of the system.

Some compromise in selectivity of the filter is necessary with this class of instrument, since reasonably high light intensities are required. For this reason apparent deviations from Beer's law may arise which are not ascribable to the absorbing system itself. In most cases empirical calibration is recommended. These instruments are widely used in clinical work (Figure 109), as well as many other "colorimetric" analyses. A collection of methods employing this instrument has been published (*132*).

In general, the single-cell type, if it employs barrier-layer cells, must use relatively nonselective filters in order to get sufficient light or very delicate high-sensitivity galvanometers. In the latter case it is still possible to measure light resolved by a monochromator (*197*).

Single-Cell Emissive Type. The use of a phototube, usually with amplification, has not met with extensive commercial use, although many sensitive circuits have been de-



Courtesy, E. Leitz, Inc.

FIGURE 103. LEITZ-LEIFO PHOTOMETER AT METROPOLITAN LIFE INSURANCE CO.

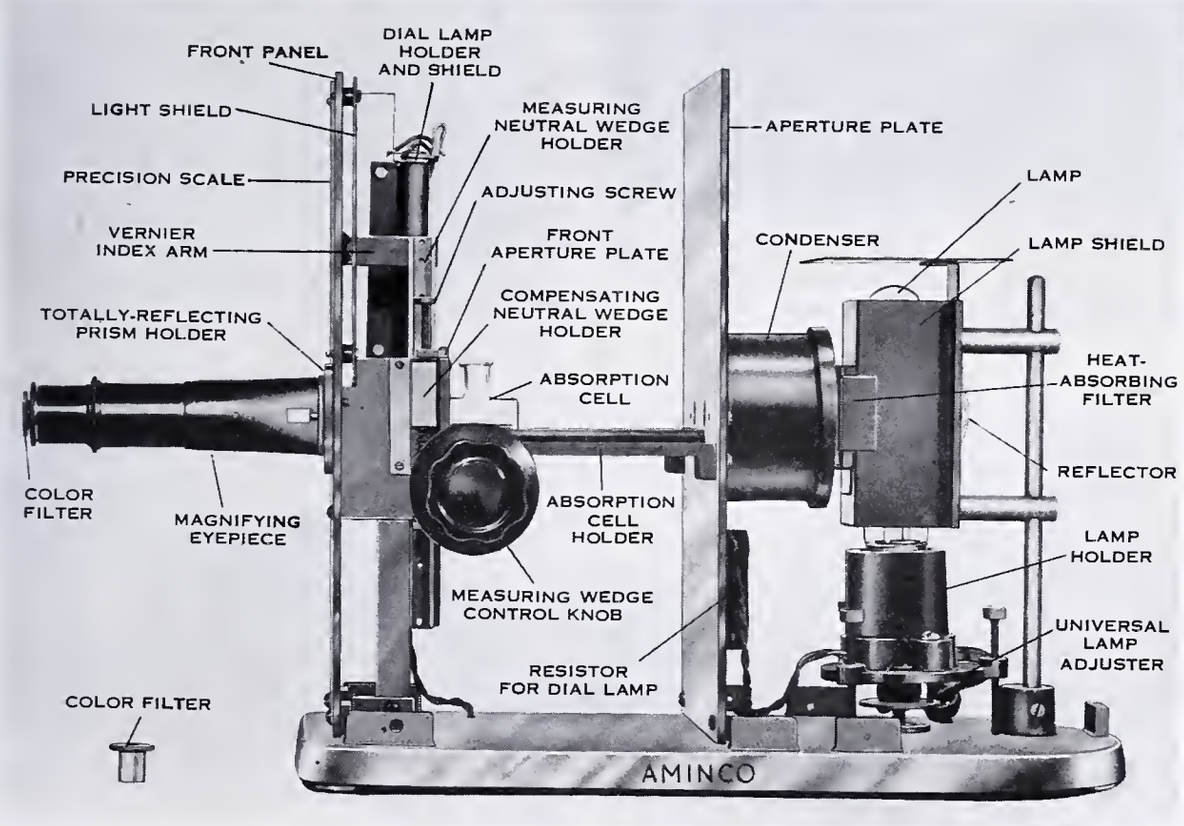
scribed (*196*, *222*). There still seems to be some objection to the inherent complication of phototube-amplifier combinations, but present trends in electronics already indicate a degree of reliability, extraordinary sensitivity, and good stability from this combination. Without going into the endless intricacies of this field, it may be mentioned that three relatively recent developments will exert an enormous effect on this class of photometers and probably stabilize practice for some time to come.

1. Electronic stabilization of light sources (*196*).
2. Inverse feedback amplifiers of extraordinary stability (*222*, *272*).
3. Multiplier-type phototubes such as the recent R. C. A. 931.

Twin-Cell Type. These may be of the barrier-layer or phototube class and in the latter case may or may not use amplification. Compensation can be attained optically or

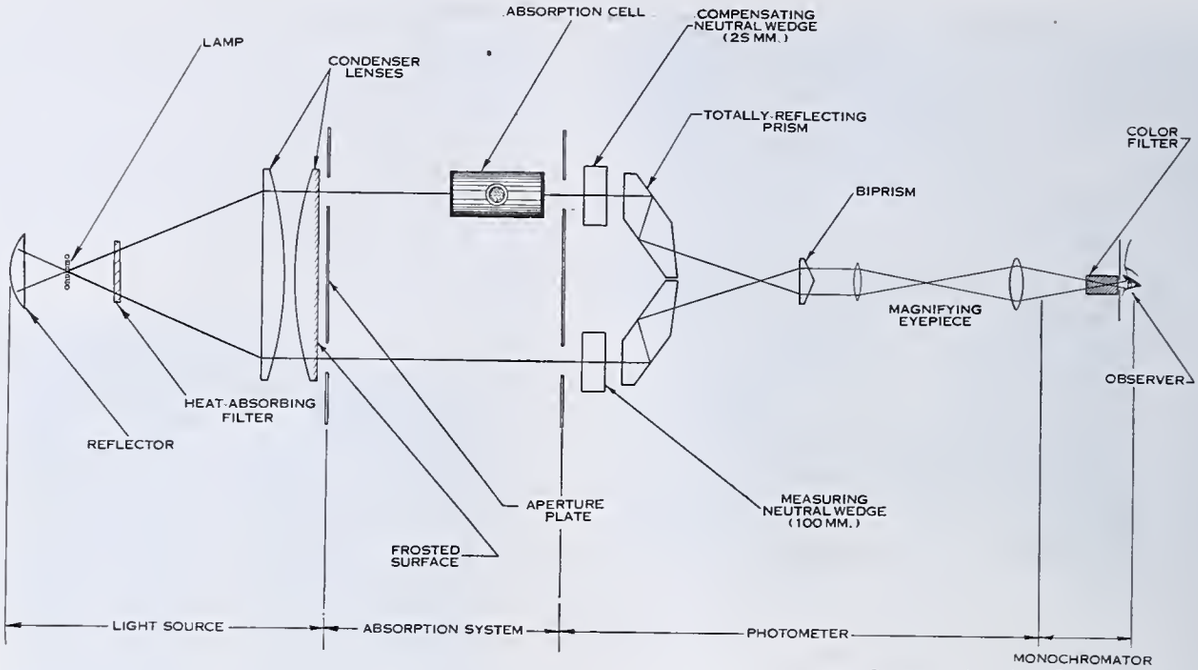
Courtesy, American Instrument Co.

FIGURE 104. WEDGE-TYPE PHOTOMETER



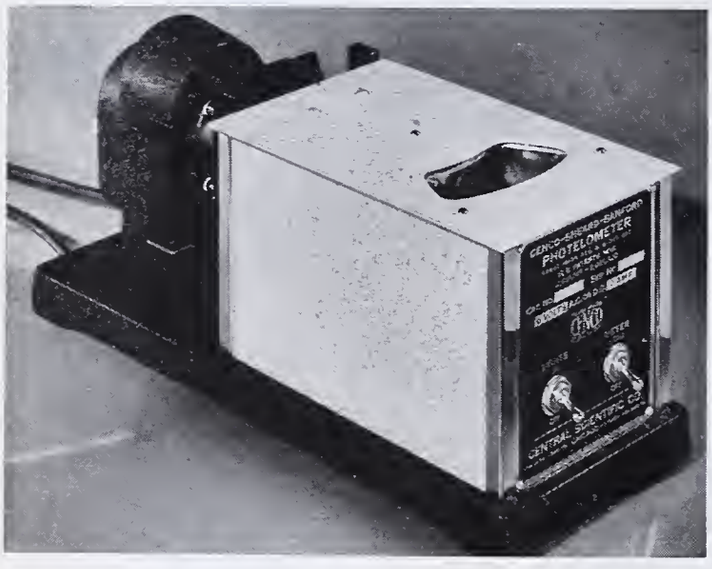
Courtesy, American Instrument Co.

FIGURE 105. WEDGE-TYPE PHOTOMETER



Courtesy, American Instrument Co.

FIGURE 106. WEDGE-TYPE PHOTOMETER



Courtesy, Central Scientific Co.

FIGURE 107. CENCO-SHEARD-SANFORD PHOTELOMETER

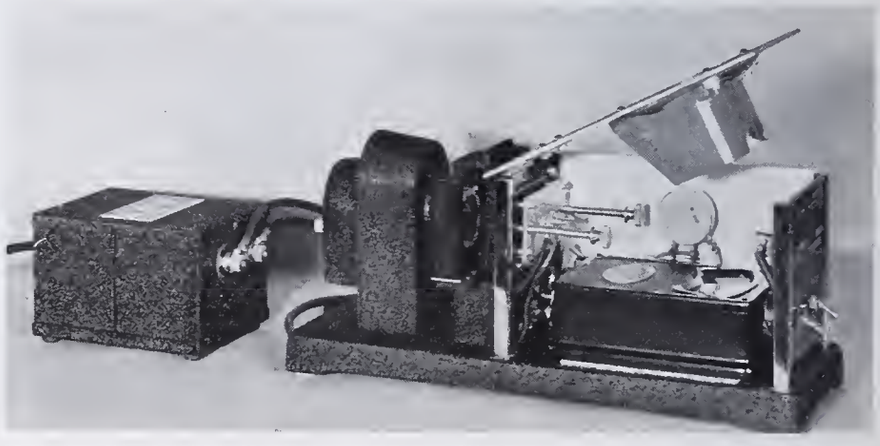
photocurrent in the measuring cell is restored to its original value, rather than decreasing the output of the comparison cell. This is a compact and carefully designed instrument worthy of the traditions of its manufacturer.

In Figure 111 is shown another twin-cell photometer using phototubes and a compensating electrical circuit. This circuit compensates for source variations and reads directly in per cent transmission, for which a decade resistor network is used. The circuit has been described by its inventors and representative results have been published (*252*), and the theory of balance has been discussed in relation to similar circuits (*196*).

The photoelectric equivalent of the Aminco Wedge photometer which uses electrical compensation is shown in Figures 112, 113, and 114.

A comprehensive review of this subject is badly needed, particularly with respect to circuit analysis and the discussion of the newer electronic developments. In general, no very high degree of photometric precision is obtained in photoelectric photometers; the fact that most of the "colorimetric" chemical methods are so very sensitive and produce measurable "color" with minute amounts of substance often gives a false sense of precision and sensitivity to the measurement. On the other hand, it is interesting to compare these electrically and source fluctuations may or may not be cancelled, depending upon the method used (*196*). A higher differential sensitivity may be expected because the main output of each cell is canceled and only the differences due to absorption are measured or compensated for. Two examples of practical instruments will be mentioned.

The Spekker absorptiometer (Figure 110) employs a light source and two barrier-layer cells connected in opposition through a galvanometer. The absorption in the sample cell is compensated by opening a diaphragm until balance is restored. The drum controlling the diaphragm is calibrated in extinction units. Compensation is made in the absorbing side and not in the comparison beam, as is often done. With this arrangement the



Courtesy, Central Scientific Co.

FIGURE 108. SIDE INTERIOR VIEW OF PHOTELOMETER

Courtesy, Central Scientific Co.

FIGURE 109. USE OF PHOTELOMETER IN CLINICAL LABORATORY

methods with spectrographic techniques, where a precision of 2 to 3 per cent is generally considered excellent. In some cases the range of sensitivity for a given constituent is about the same, although in others the spectrograph is far more sensitive.

APPLICATIONS. A large number of applications of the visual types of photometers are classified with references and titles in the works of Urbach (*285A*), Krebs (*162*, *311*), and Heilmeyer (*114*), and in bulletins by Carl Zeiss, E. Leitz and Co., and the American Instrument Co. In addition, the treatises on colorimetry by Yoe (*309*) and Snell (*262*) contain specific reference to the use of photometers, both visual and photoelectric. All other analyses listed by them are suitable for use with any of these instruments, although if they were developed for use with a Duboscq colorimeter or comparison tubes, the necessary information relating to proper filters, etc., will be lacking (see also *99*).

The principle of photometric titration has been employed from time to time in order to substitute titrimetric precision for a single photometric measurement, or better stated, to combine the photometric technique with that of titration. The arrangement shown in Figure 115 has been used in the author's laboratory for this purpose. The apparatus has somewhat wider utility, since it is automatically recording and therefore suited to follow slow changes in transmission.

It consists of an electronically regulated light source constant to 0.02 per cent, which illuminates the slit of a monochromator. A titration cell receives the monochromatic light which, after traversing the cell, strikes a vacuum phototube. The phototube and preamplifier are in a light-tight case to the right of the titration vessel. The photocurrent is fed to a high-gain inverse-feedback amplifier which delivers a linear 10-milliampere output for very low levels of illumination (exit slit of monochromator $\simeq$ 1 mμ = 10 Å.). The stability and linearity of this amplifier are high enough to require an output meter accurate to 0.1 per cent, which accounts for the precision standard meter visible in the foreground. For recording purposes, the output current may be sent through a standard series resistor box, the terminals of which are connected to a Micromax recording potentiometer.

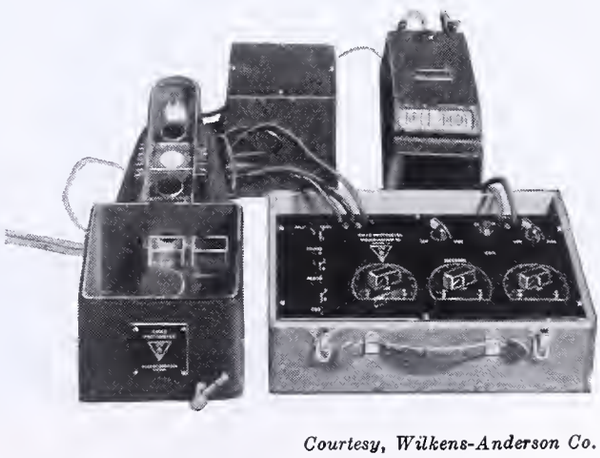
Some typical photometric titrations to test Beer's law are shown in Figure 116. The readings as well as the records are transmission values and have been recalculated to extinction values, and corrections for dilution due to the addition of reagent have been made. This equipment is primarily for research on systems of analytical interest—i. e., to test Beer's law, study equilibria and rate of reaction, and establish stoichiometric ratios and thereby ascertain the nature of the light-absorbing entity.

"Colorimetric"

This term, as understood by the chemist (*196*), refers to the analytical method whereby the concentration of a substance is determined by means of its color or by a color produced upon the addition of a suitable reagent. It is one of the most widely used analytical techniques and is primarily suited for very small amounts or concentrations. The methods of measurement vary from simple comparison in test tubes or Nessler tubes to the use of elaborate instruments. In the extension of the technique to more and more refined measurements it reduces to a photometric or a spectrophotometric problem. The compilation of colorimetric methods is tremendous and many treatises discuss the various instruments in detail (*80*, *262*, *309*). In many respects colorimetric methods compare very favorably with other analytical techniques such as the spectrograph, polarograph, etc., in sensitivity and precision.

Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.

FIGURE 110. SPEKKER ABSORPTIOMETER



Courtesy, Wilkens-Anderson Co.

FIGURE 111. TWIN-CELL PHOTOMETER

THEORY. Colorimetric matching involves the comparison of a colored sample, with or without instrumental aid, with a reference sample which may be an artificial reference or one which is identical in nature but of known concentration. In general, white light is used for the matching and some degree of hue discrimination as well as intensity matching is involved. If colored or filtered light is used, the technique is more properly called filter photometry. One principle of comparison involves the choice of suitable depths of solution (standard and unknown) until the two colors match, and under these conditions the respective concentrations are inversely proportional to the corresponding depths.

$$c_1 l_1 = c_2 l_2$$

This is a consequence of the Lambert-Beer law discussed above.

Courtesy, American Instrument Co.

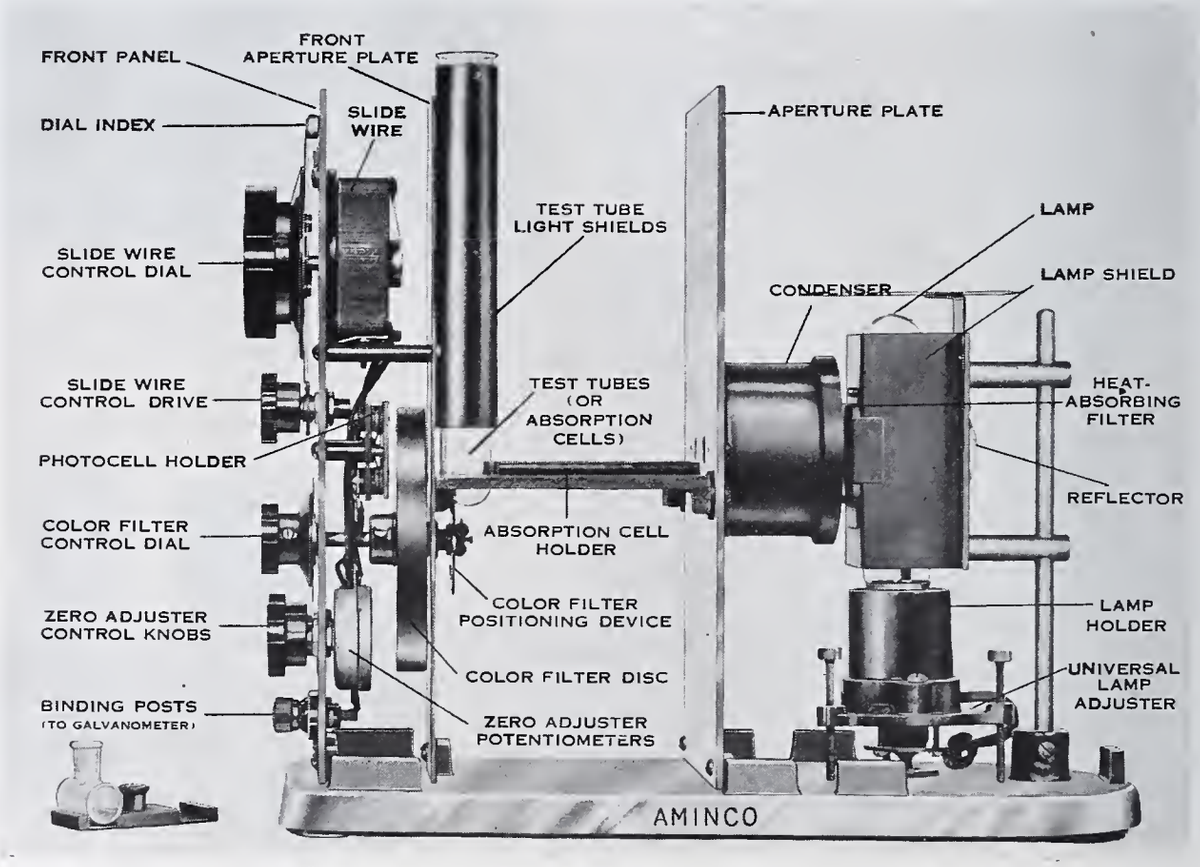
FIGURE 112. PHOTOELECTRIC PHOTOMETER

Special cases arise in which the equilibrium between two colored species is to be determined, as, for example, in some pH measurements. These are discussed below.

INSTRUMENTS. No discussion of test tube or Nessler tube comparison is called for here, since these are not instrumental methods, although numerous aids, such as comparator blocks or roulette comparators, are very useful accessories.

A very convenient comparator, shown in Figure 117, is one of numerous models manufactured by the company. The sample is placed in one of the fused-glass rectangular tubes and a similar tube is filled with distilled water. An appropriate color wheel or disk is then introduced, which upon rotation introduces nonfading glass color disks into the optical path. When one is found which matches the sample, its designating number is read at the aperture on the lower right. A simple eyepiece with prism brings the two color fields in juxtaposition. The comparison tube also serves to compensate for any turbidity or background color in the sample. Color disks are provided for twenty-four different pH indicators, for iron, ammonia, nitrite, chlorine, manganese, dissolved oxygen, lead, phosphate, silica, sulfides, and many other colorimetric methods. Color disks are also available for special classes of work, such as the color determination of petroleum oils and petrolatum, color of varnish, resins, lacquers, etc. Instruments of this sort are well suited for rapid, reasonably precise tests in routine or field work.

A familiar class of instrument is the Duboscq colorimeter, of which there are many models and types (*197*, Figure 60). Figures 118 and 119 illustrate two models that find extensive use in industry and in the clinical field. Modifications of the general principle adapt this class of instrument to special problems as, for example, the hemoglobinometer, the microcolorimeter, and the hydrogen-ion colorimeter. In the latter, unique provision is made for the measurement of equilibrium between the two colored forms of an indicator. The principle



Courtesy, American Instrument Co.

FIGURE 113. PHOTOELECTRIC PHOTOMETER

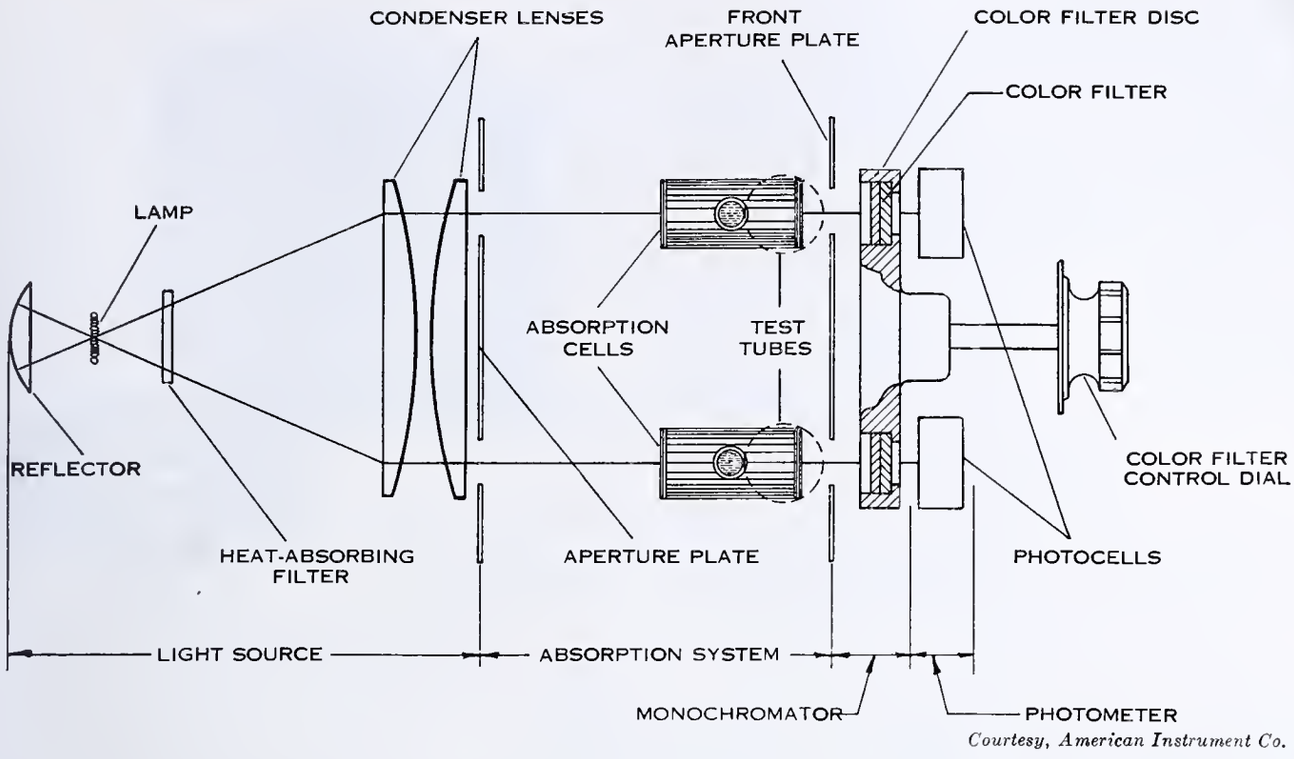


FIGURE 114. PHOTOELECTRIC PHOTOMETER

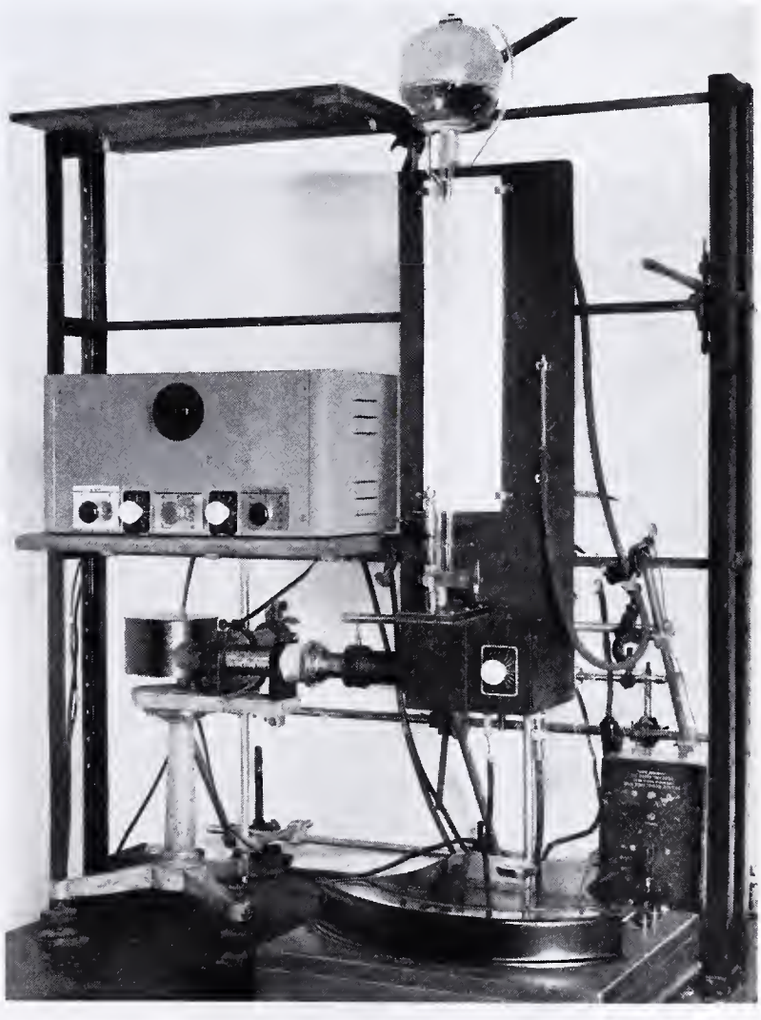
is described in detail by Clark (*40*). In the expression relating pH to the apparent dissociation constant, K', of an indicator

$$\text{pH} = \text{p}K' + \log a/(1 - a)$$

the fraction, a, of the indicator which is in one of the colored forms and the other, $1 - a$, can be measured colorimetrically, and hence, knowing pK', the pH can be computed.

The hydrogen-ion colorimeter uses twin cups on either side. On the compensating side, one cup contains indicator in the acid form, the other cup in the basic form. The total depth is held constant, but by moving the inner cup, any hue ranging from the complete acid color to the full basic color can be obtained and the cup settings yield the ratio $a/(1 - a)$. Log tables of these ratios are provided, and the appropriate values are simply added to the correct pK' for that indicator.

APPLICATIONS. There are so many applications of this method and in general they are so familiar that it is pointless to enumerate even typical examples (*6*). In clinical chemistry a very appreciable fraction of all analyses are conducted in this manner, because the method is rapid, requires very little material, and is sufficiently precise for the purpose. A tremendous amount of research and ingenuity has gone into the development of colorimetric chemical procedures; indeed, many substances by nature are not addicted to the formation of colored compounds and it has required clever indirect methods to bring them within the scope of this subject. Instruments for the purpose are relatively cheap, convenient, and versatile. The competition which photoelectric photometers have occasioned is real, but the simplicity and directness of the colorimeter will continue to be attractive for some time to come.



Courtesy, New York University

FIGURE 115. MANUAL AND RECORDING PHOTOMETRIC TITRATION APPARATUS

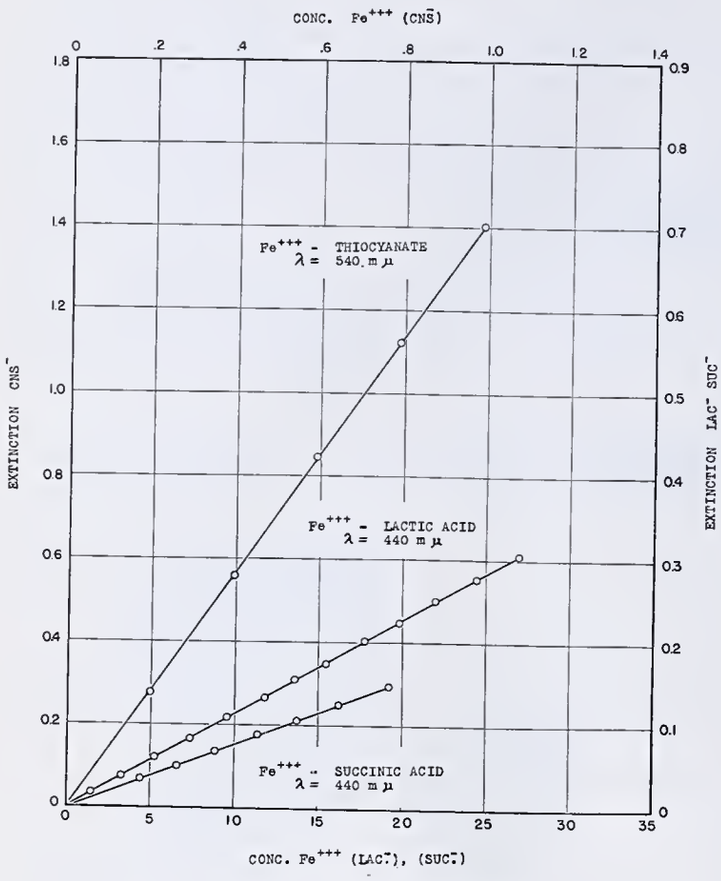


FIGURE 116. PHOTOMETRIC TITRATIONS

Courtesy, Hellige, Inc.

FIGURE 117. COLORIMETRIC COMPARATOR

Fluorometric

Early studies in fluorescence were devoted to the elucidation of the phenomenon, the connection between chemical constitution and fluorescing ability, and a study of the factors which affect the phenomenon. It is an extremely complex phenomenon and although known, by a few examples, to the ancients it was often demonstrated but rarely used. Analytical uses were proposed a long time ago and several treatises (*48*, *220*) list hundreds of tests and analytical schemes. Although the phenomenon is common, it is more frequently associated with substances of limited analytical interest. Nevertheless, there are enough cases to make it worth while, and especially in the case of vitamin assay, important enough to stimulate the development of suitable instruments.

THEORY. The fundamentals of the phenomenon are discussed at great length in two monographs (*79*, *219*). Upon absorption of light, a fluorescing substance re-emits the energy, generally at wave lengths longer than the exciting radiation (Stokes' law). In many systems and at extremely low temperatures definite energy states in the emitting system can be recognized, but at higher temperatures and especially in solution, the fluorescence spectrum is a broad "washed-out" band. The theoretical requirements of the quantum theory are obeyed in the energy distribution, as shown by Wawilow (*292*). The energy re-emitted is not directly proportional to the concentration—i. e., there are self-quenching effects. What is more important is the fact that many ions exert a powerful quenching effect on the fluorescence. The various effects which have an important bearing on analytical applications can be stated briefly. The specific fluorescence depends on (1) concentration of the fluorescing substance, (2) wave length of excitation, (3) pH, (4) temperature,

Courtesy, Bausch & Lomb Optical Co.

FIGURE 118. DUBOSCQ COLORIMETER

(5) presence of foreign ions, and (6) presence of other fluorescing substances.

The effect of pH is so pronounced that fluorescent substances have been used as "ultraviolet" indicators for titrations (*55*), although in view of (5) this is often attended by complications. The effect of foreign ions has been investigated in detail (*144*, *198*, *296*) and the information should be applicable to its influence on analytical uses of the method.

The general procedure consists in illuminating the sample with monochromatic ultraviolet light and measuring, either visually or photoelectrically, the visible fluorescent light which is emitted. As a whole, a true photometric method is to be preferred—that is, to measure the fluorescence per unit intensity of the exciting radiation. In some cases a choice of the exciting radiation with respect to wave length permits an analysis even in the presence of other substances which fluoresce.

Instruments. The Pulfrich and Leifo photometers, previously mentioned, are available with suitable accessories for precise fluorescence measurements. Photoelectric fluorometers are becoming increasingly popular and already enjoy wide use, particularly in the vitamin field. The Hilger instrument shown in Figure 120 is an extension of the photometer absorptiometer, Figure 110. An external, sensitive galvanometer is used to indicate balance, which is achieved by optical compensation. One well-known instrument has been described (*197*, Figure 70) and one of its applications is illustrated in Figure 121. Another, using a phototube and stable amplifier, is shown in Figures 122 and 123. Excitation is provided by a mercury arc and suitable filters. Very simple controls adjust zero setting, a standard deflection in terms of a reference sample and a control for balancing out small initial deflections corresponding to the blank. Sensitivities several times as high as those obtainable with the simpler cell-galvanometric arrangement are claimed; on the basis of current practice in electronics this is a reasonable claim.

Applications. These include the determination of vitamin B_1 (thiamin, *117*), B_2 (riboflavin, *117*), and all naturally fluorescing substances such as quinine, other alkaloids, oils, waxes, and proteins. Methods whereby nonfluorescing substances can be chemically treated to produce a fluorescing substance are discussed in reference works (*48*, *220*) and by White (*298*). Mention should also be made of the semianalytical applications in forensic work for the detection of adulterants, forgeries, and spots or suspected artifacts. These are usually accomplished by visual inspection or photography.

Turbidimetric and Nephelometric

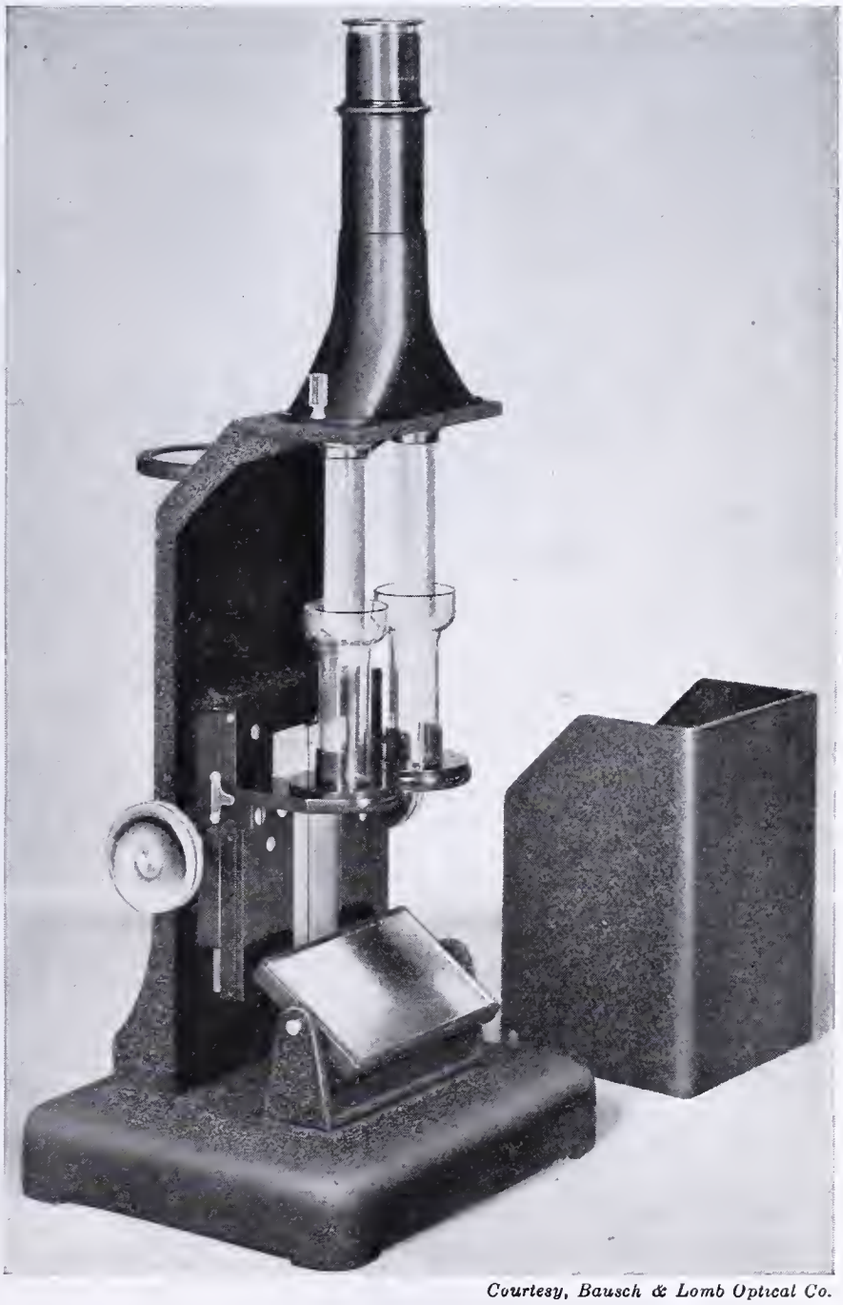
The optical properties of finely divided particles or disperse systems have some interest for the analytical chemist but considerably more interest for the colloid chemist who is interested in learning more about the nature of these dispersions. Since such dispersed systems scatter light, a suitable measurement of the light scattering may be used to determine the amount of dispersed material. Three methods may be used:

1. Light which has traversed a turbid medium will be scattered to some extent and there will therefore be a decrease in the intensity of the emergent beam.
2. The intensity of the light which is scattered laterally may be compared with a suitable reference and the procedure is then known as nephelometric.
3. An accurate measurement is made of the ratio of intensity of the scattered to the incident light by means of a Tyndallometer. The principle and design of this instrument were described by Mecklenburg and Valentiner (*189*); it is manufactured by Schmidt and Haensch of Berlin.

Light scattering is an extremely complex phenomenon and the theory of the phenomenon was given in detail by Lord Rayleigh (*294*), according to which the intensity of the scattered light is given by:

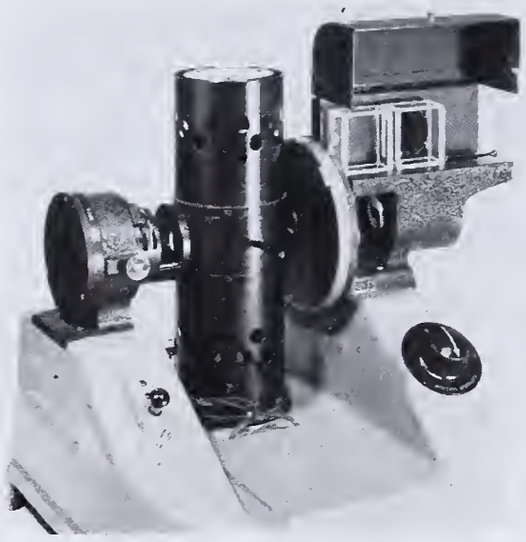
$$\frac{I}{I_0} = K\frac{Nv^2}{\lambda^4}\sin^2\alpha$$

where I is the intensity of the scattered light viewed at an angle α to the primary beam of intensity I_0; N is the number of particles of volume v, and λ is the wave length.



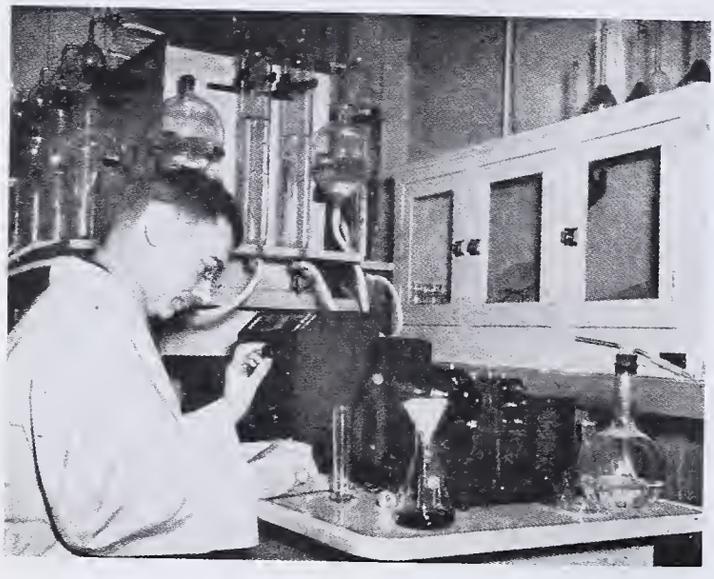
Courtesy, Bausch & Lomb Optical Co.

Figure 119. Duboscq Colorimeter



Courtesy, Jarrell-Ash Co. and Adam Hilger, Ltd.

FIGURE 120. SPEKKER FLUOROMETER



Courtesy, Pfaltz & Bauer, Inc.

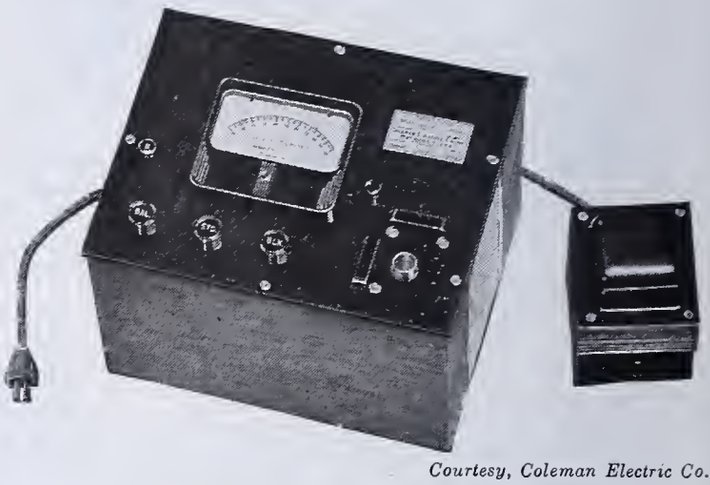
FIGURE 121. USE OF FLUOROPHOTOMETER

Of principal analytical interest is the fact that the measurements will be affected by (1) manner of mixing sample and reagent, (2) ratio of the concentrations, (3) rate of mixing, (4) rate of development to maximum turbidity, (5) temperature, (6) stability of the dispersion, and (7) effect of foreign substances—electrolytes and nonelectrolytes.

It is also assumed that the dispersed phase is colorless, for if colored dispersions are produced there is the added complication of selective light absorption.

A discussion of these complexities and a theoretical and practical treatment are available (*104*, *153*, *295*, *309*, Vol. II).

INSTRUMENTS. Suitable instruments for the comparison of relative turbidities range from test-tube comparator blocks to more elaborate means of photometric comparison. An example of a very useful turbidimeter for sulfate determinations is shown in Figure 124. The estimation of turbidity with this instrument is obtained by measuring the depth of the suitably prepared dispersion through which one can still observe the filament of a small lamp. A plot of the thickness of liquid against the concentration of the dispersed phase yields a smooth curve, as shown in Figure 125. The photoelectric equivalent of this instrument is shown in Figure 126; the advantage of the second form lies in the more objective nature of the setting. Corresponding calibration curves for this instrument are shown in Figure 127. Both instruments have found wide application for this class of analyses, particularly in sulfate determinations, with which they are conveniently used in connection with the Parr sulfur bomb (Figures 128 and 129).



Courtesy, Coleman Electric Co.

FIGURE 122. PHOTOFLUOROMETER

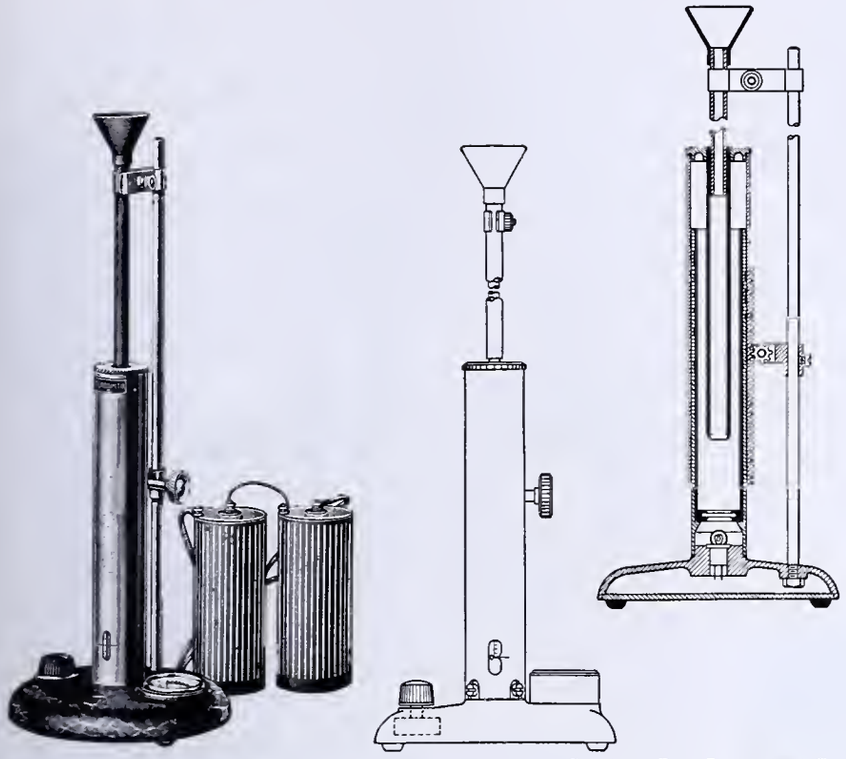
Another approach to the same problem is illustrated in the Hellige instrument (Figures 130 and 131), in which a twin photometric field results from the lateral illumination of the suspension and a comparison beam which is reflected upward through the system. The comparison beam may be adjusted by means of a precision slit (*S*, Figure 132).

Many of the commercially available photoelectric photometers are adaptable to turbidimetric measurements. In practically all cases such use implies empirical calibration, primarily to suit the optics of the system but more especially because of the great influence of the various factors noted above.

Several sources describe alternative optical approaches,

Courtesy, Coleman Electric Co. and Continental Can Co.

FIGURE 123. ELECTRONIC PHOTOFLUOROMETER IN CHEMICAL RESEARCH



Courtesy, Parr Instrument Co.

FIGURE 124. TURBIDIMETER

some of them based on the fact that the light scattered by such media is almost completely polarized.

APPLICATIONS. The method is particularly suited for the determination of small quantities of any substance which can be "precipitated" under conditions that will maintain a stable and uniform dispersion. An extensive list of such applications, along with detailed directions, is to be found in Yoe's monograph (*309*, Vol. II) and some later applications, particularly by photoelectric means, have been mentioned by the author (*196;* see also *27*, *102*, *267*). An interesting extension of the technique has been described by Goodhue (*98*), who developed a turbidimetric titration scheme for the determination of small amounts of nicotine (see also *15*). There have also been many applications of these methods to problems of no particular analytical interest, such as the determination of sedimentation rates.

What has been said about these measurements applies also to dispersions in gases and has proved very useful in the examination of very dilute smokes and vapors.

Refractometric

The refractive index of a medium is an important constant and has useful analytical applications. It can be measured in any region of the spectrum from electric waves to the shortest x-rays, and in all states of aggregation, solid, liquid, or gas. Although it is an unspecific property, in many instances its numerical value is an important identifying characteristic which, in connection with other properties, defines the system. It finds many uses in technical analysis for control of purity and the analysis of simple binary mixtures.

THEORY. The refractive index, n, expresses the ratio of the velocity of light in the two media which form the boundary. The law of Snell expresses n as the sine of the angle of incidence divided by the sine of the angle of refraction or

$$n = \frac{\sin i}{\sin r} \qquad (4)$$

The specific refraction may be expressed according to Gladstone and Dale as

$$R = (n - 1)/d \qquad (5)$$

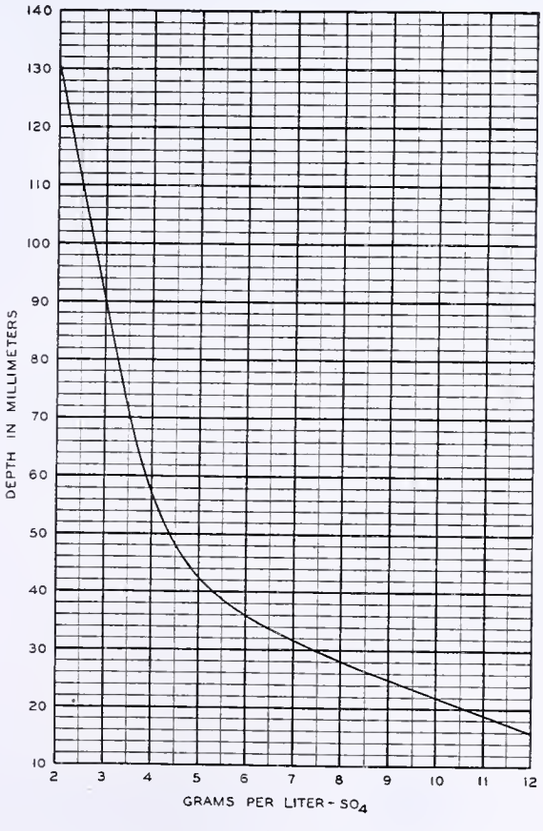
where d is the density, or somewhat better by the Lorenz-Lorentz equation

$$R = \frac{n^2 - 1}{n^2 + 2} \times \frac{1}{d} \qquad (6)$$

The product of this quantity and the atomic or molecular weight yields the atomic refraction or molecular refraction. The sum of the atomic refractions is equal to the molecular refraction, provided the necessary corrections are made for the binding or linkages. The contribution of double and triple bonds and conjugated linkages has been evaluated, and in the early applications these data are very useful in establishing the structure of complex organic compounds. Small constitutive variations in these corrections are now recognized and the problem is not so simple as the early considerations indicated.

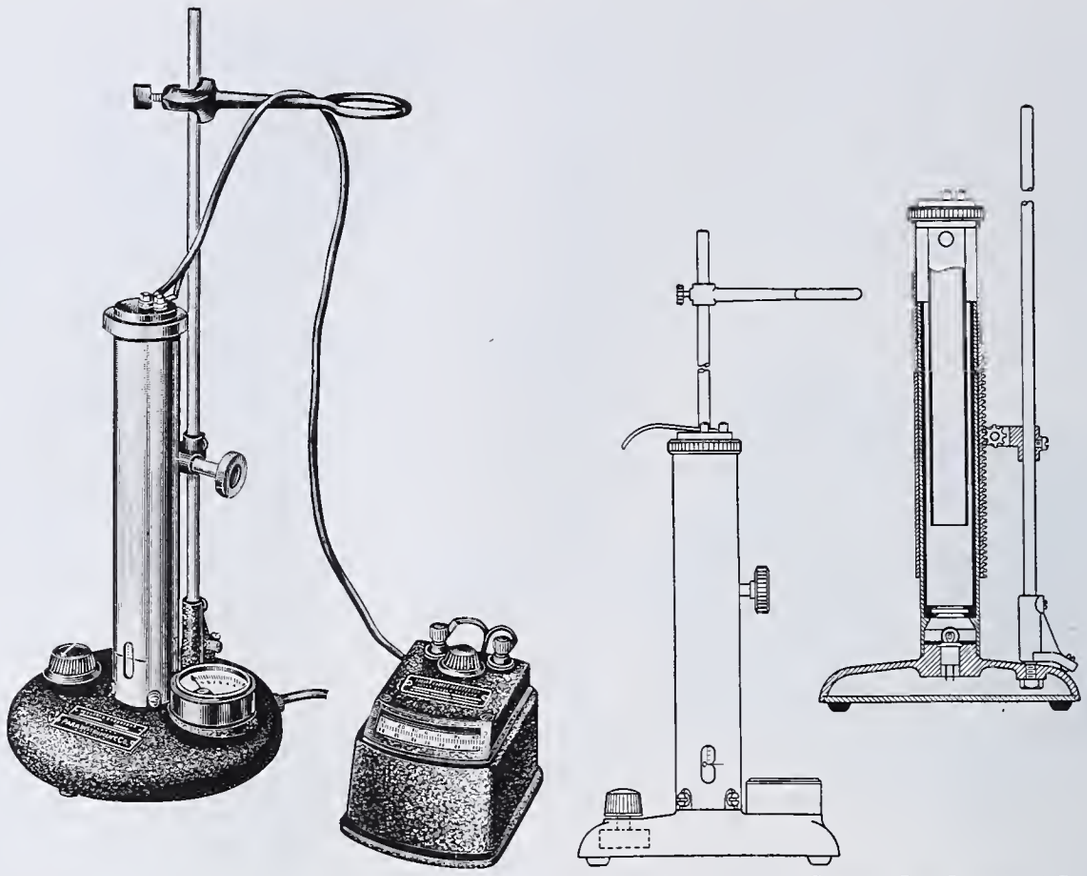
The change in refractive index with wave length is known as the dispersion, and this quantity is equally important and useful (*289*, *302*). It is usually expressed as $(n_F - n_C)$ where the subscripts refer to the hydrogen lines, F = 4861 Å. and C = 6563 Å.

The connection between Equation 6 and the total polarization, P, as determined from the dielectric constant is discussed under the latter heading.



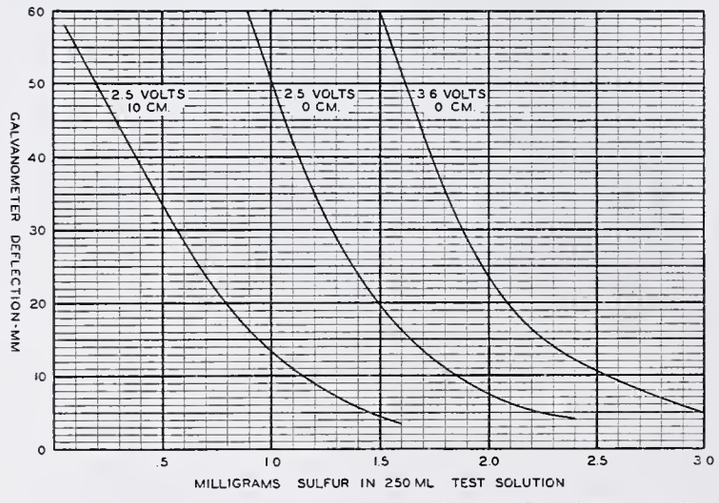
Courtesy, Parr Instrument Co.

FIGURE 125. TYPICAL CALIBRATION CURVE FOR VISUAL TURBIDIMETER



Courtesy, Parr Instrument Co.

FIGURE 126. TURBIDIMETER, PHOTOELECTRIC TYPE



Courtesy, Parr Instrument Co.

FIGURE 127. TYPICAL CALIBRATION CURVE FOR PHOTOELECTRIC TURBIDIMETER

The refractivity of mixtures might be expected to follow a simple mixture law of the type

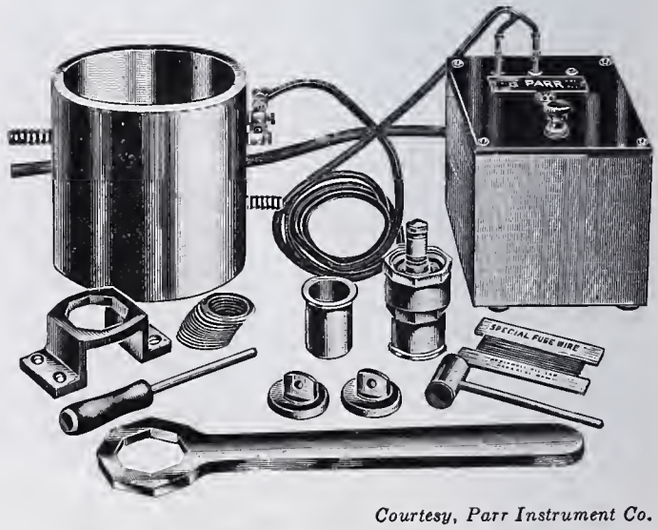
$$100\,R = P_1R_1 + P_2R_2 + \ldots\ldots\ldots$$

where the P's represent per cent of the components and the R's the corresponding refractivities, but this assumes no interaction of the molecules; and to the extent that the solutions deviate from ideality one can expect this to appear in the total refraction. In most cases a mixture is analyzed by reference to the refraction of mixtures of known composition.

Both refractive index and dispersion vary with the temperature; in solutions the temperature coefficient is usually large and negative in sign.

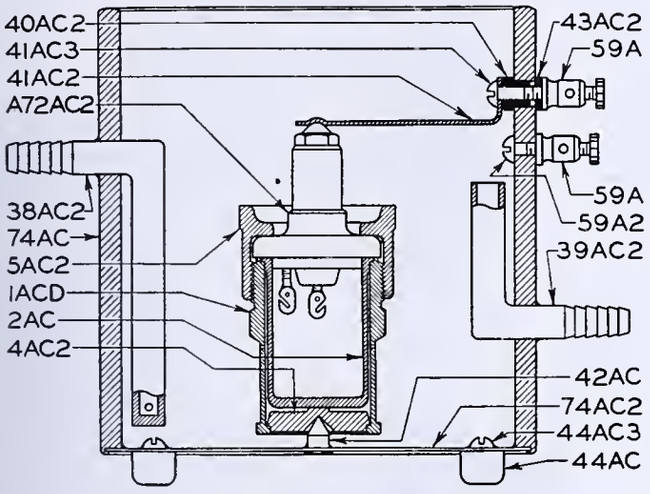
INSTRUMENTS. Many simple devices may be used for a rough measurement of n (*152*), and the ordinary microscope may be used for this purpose if no refractometer is available. These methods are discussed in standard works on optics (*294*). Four general classes of instrument will be mentioned.

Abbe Refractometer. This instrument is direct-reading in refractive index for the sodium lines, n_D, with a range of about 1.3 to 1.7 and can be estimated to ± 0.0001. Solids or liquids within this range can be measured. For liquids only a drop is necessary



Courtesy, Parr Instrument Co.

FIGURE 128. COMPLETE PEROXIDE SULFUR APPARATUS



Courtesy, Parr Instrument Co.

FIGURE 129. SULFUR BOMB AND WATER BATH

and it is held between two prisms. The prism mountings are hollow, permitting the use of circulating water from a thermostat to maintain constant temperature. The principle depends upon the critical angle of total reflection which is determined by n of the liquid and the constant, n, of the contiguous prism. White light may be used as the source and an Amici prism is used to cancel dispersion effects. When the prism is rotated to produce an achromatized shadow edge, the sodium line passes through undeviated. The angular setting of the Amici compensator enables one to obtain the dispersion. Tables are usually supplied for this information. Several examples of this instrument (*196*, Figure 53) are illustrated in Figure 133. Special scales can be provided for an Abbe refractometer for use as a direct butyrorefractometer, sugar refractometer, etc.

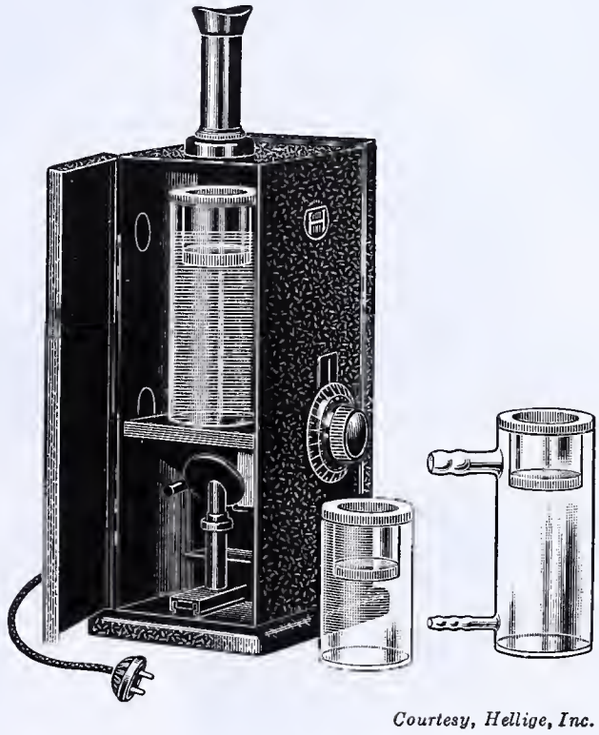
Pulfrich Refractometer. This instrument also employs the total-reflection principle and is particularly suited for the determination of dispersion values. It is not direct-reading; the angle of the deviated ray is referred to tables from which n may be obtained. No achromatizing arrangement is provided, so that full dispersion is observed in the eyepiece. In its ordinary form n can be determined to ± 0.0001, but more elaborate forms of the instrument have reduced the uncertainty to ± 0.00001. This instrument is manufactured by Carl Zeiss and by Adam Hilger. A full description is given in most texts on optics and physical chemistry.

Dipping Refractometer. This is a limited-range instrument with correspondingly higher precision. In order to cover the entire range, interchangeable prisms are provided and as many as a half dozen may be required. A typical instrument is shown in Figure 134 and schematically in Figure 135. An arbitrary scale is mounted in the eyepiece and a rotating drum makes it possible to read the position of the dividing line on the scale to tenths of a division. The average precision is of the order of ± 0.000035. A compensating prism is also used to achromatize the shadow edge. It is rotated by the knurled ring near the prism. The prism is usually dipped into a sample cup, but where limited amounts are available, a special cup may be used containing an auxiliary prism which nearly engages the hypotenuse face of the instrument-prism. A thin film of the liquid is thus caught between the two.

Interferometer. In a refractometer, the refractive index itself is measured, whereas with the interferometer the difference in refraction of two liquids or two gases is determined (*109*, *294*). This is the usual case, although it is possible to calibrate an interferometer in absolute terms, using monochromatic light of known wave length. Since a change in refractive index results in a change in the velocity of light through the medium, it will be apparent from the definition of n that to measure n we need but measure the difference in velocity. Light coming from a common source and traveling in two separate beams along the same distance will arrive in phase at any point, but if one path is through a medium of slightly higher refractive index, this is equivalent to a virtual change in distance traversed and the two beams will be out of phase and show interference fringes. The phase difference can be restored by moving a compensator plate driven by a slow-motion screw.

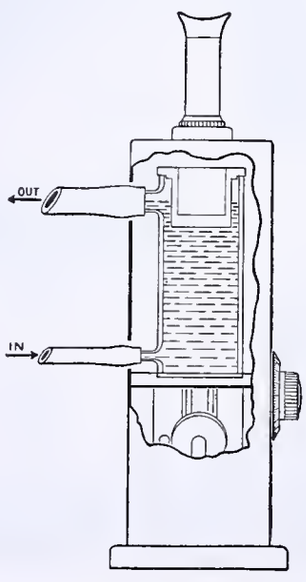
Any interferometer can be used to demonstrate this principle, but for exceedingly small differences it is necessary to bring the two arms together in order to rule out any appreciable temperature differences. Under the best conditions, values a thousand times smaller than those measurable with a refractometer can be determined.

For example, in the case of gases with a 100-cm. chamber, the error is of the order of 2×10^{-8} n. For liquids in 8-cm. depth about $\pm 2.5 \times 10^{-7}$ and at 1-mm. depth about $\pm 2 \times 10^{-5}$. With the gas interferometer shown in Figure 136, a



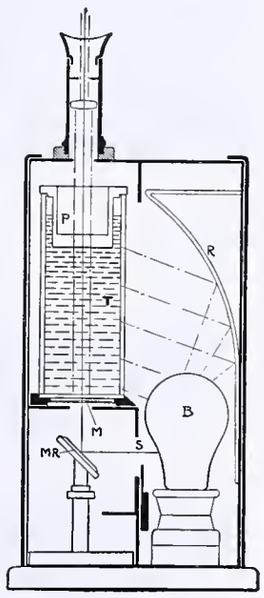
Courtesy, Hellige, Inc.

FIGURE 130. HELLIGE TURBIDIMETER



Courtesy, Hellige, Inc.

FIGURE 131. HELLIGE TURBIDIMETER FOR CONTINUOUS READING



Courtesy, Hellige, Inc.

FIGURE 132. HELLIGE TURBIDIMETER, DIAGRAMMATIC VIEW

portable instrument with a 10-cm. chamber, the error in measuring carbon dioxide, methane, or hydrogen in air amounts to about 0.1 per cent. The same instrument can be fitted with 25- and 50-cm. chambers; the errors are then 0.04 and 0.02 per cent. It is suited for rapid "on the spot" analyses of noxious fumes, carbon dioxide in expired air, or ether in the atmosphere of operating rooms (±0.4 to 0.8 gram per cubic meter).

Applications. Among the hundreds of uses a few typical ones may be mentioned. An interesting and important application (*149, 243, 249*) is illustrated in Figure 137.

The object of this particular installation is to measure the vapor concentration of toxic organic solvents in the air of a large (1032 cu. feet) experimental chamber. Air is circulated rapidly

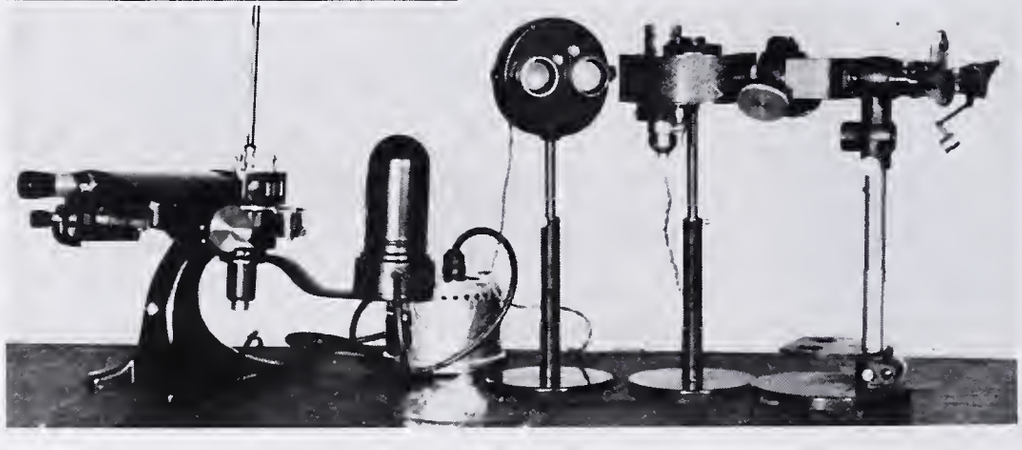
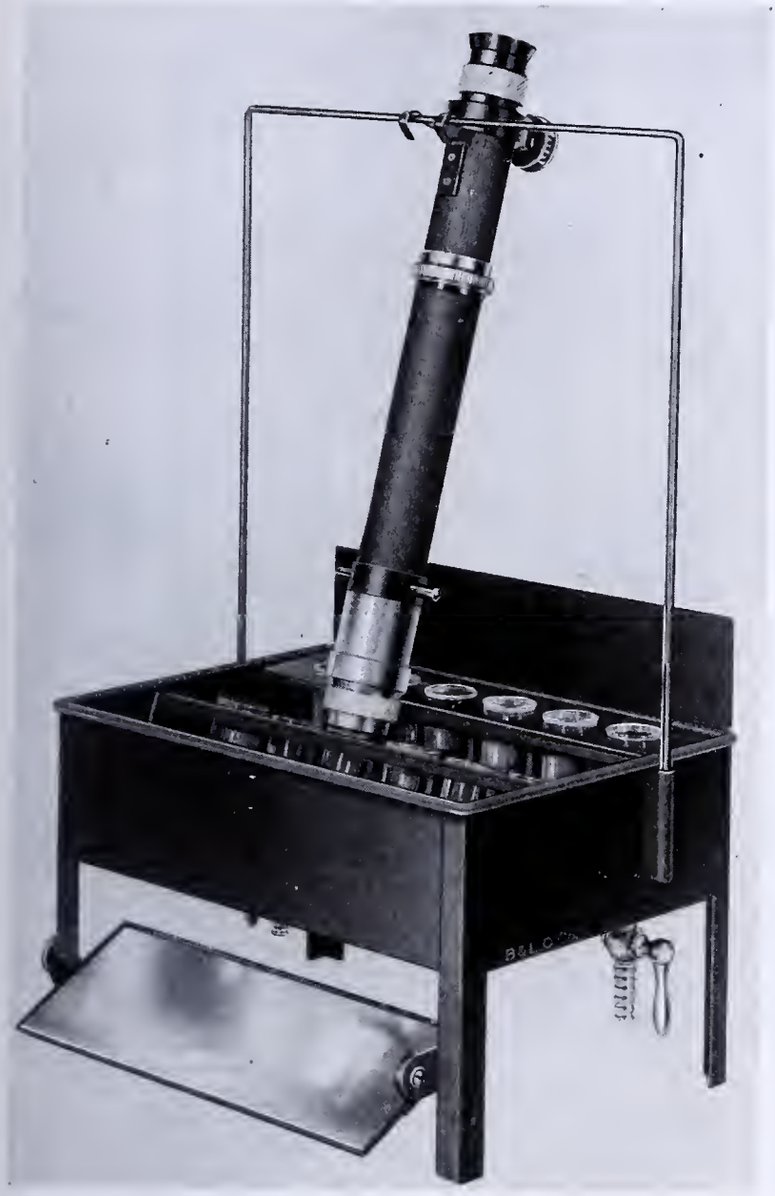


Figure 133. (*Upper Left*) Jelly-Type Refractometer for Use with a Drop of Liquid
Courtesy, Fisher Scientific Co.

(*Left Center*) Abbe Type Refractometer
Courtesy, Spencer Lens Co.

(*Lower Left*) Bausch & Lomb Precision Refractometer–Pulfrich Photometer as Used for Measurements on Sugar Solutions. *Courtesy, F. W. Zerban, New York Sugar Trade Laboratory*

(*Upper Right and Right Center*) Valentine Refractometer, Improved Abbe Type
Courtesy, Industro-Scientific Co. and Gamma Instrument Co.



Courtesy, Bausch & Lomb Optical Co.

FIGURE 134. DIPPING REFRACTOMETER

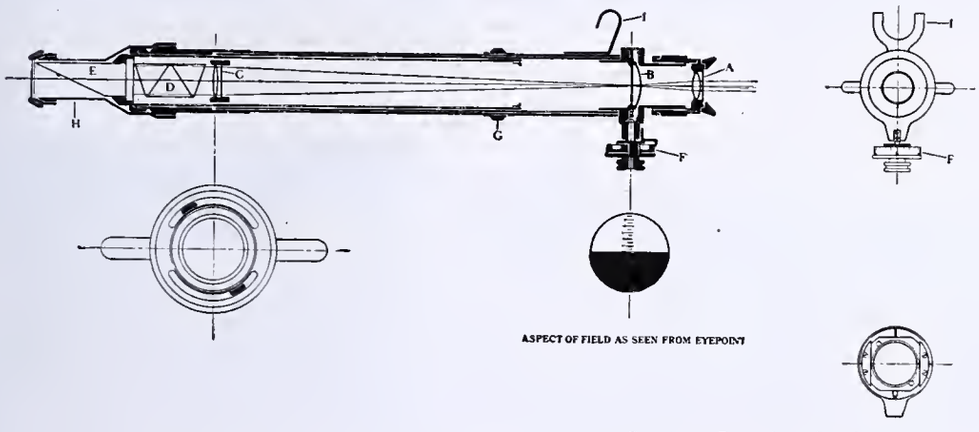
converted between the sampling ducts and the interferometer chamber. The instrument must be shielded from air currents (avoiding thermal gradients) during measurements. Concentration of toluene vapor ranging from 25 to 1000 parts per million in air can be measured and the instrument has also been used for determining the concentrations of vinyl cyanide vapors.

Further uses of the interferometer include gas analysis (*218*, *248*), the analysis of oils (*172*, *186*, *203*, *237*, *290*), fats, and waxes (*173*), and the analysis of very dilute aqueous solutions (n = 0.0004 to ±0.1 per cent) by means of a differential refractometer (*221*). A discussion of n as an analytical constant, with 92 references, has been published by Fulweiler (*83*) and a means for determining fat in chocolate by Stanley (*266*). Other uses have been practiced for such a long time that they are almost taken for granted; sugar and alcohol tables (*253*) are to be found in all manuals of food analysis (*31*, *32*) (see Figures 138, 139, and 140), and many other systems of analytical utility (*164*, *182*) are listed in International Critical Tables and related compilations (*175*, *229*).

Polarimetry

The rotation of the plane of polarized light has long been a convenient means of analysis, limited, of course, to systems containing molecules with an asymmetric atom, the most common of which is carbon, but amenable to many compounds of sulfur, tin, selenium, silicon, and pentavalent nitrogen. The technique is confined mostly to the analysis of sugars (*31*, *32*, *140*), essential oils, and alkaloids.

INSTRUMENTS AND METHODS. Representative examples of this instrument are shown in Figure 141. The polarimeter shown in Figure 142 contains all the refinements for very precise work, including preliminary purification of the light by a monochromator. A splendid example of a precision installation is shown in Figure 143. The polarimeter shown in Figures 144 and 145 is an interesting example of the utilization of that versatile product of modern times—polaroid film. This presents for



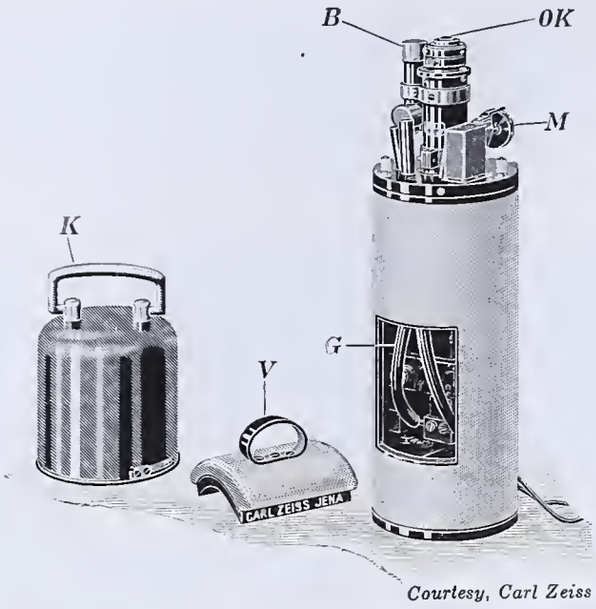
Courtesy, Bausch & Lomb Optical Co.

FIGURE 135. SECTIONAL VIEWS OF DIPPING REFRACTOMETER

by means of a fan in a set of recirculation ducts. One hundred and thirty cubic feet per minute of fresh air are supplied, which is sufficient to eliminate the building up of carbon dioxide and water vapor in the chamber and to prevent the upset of the basic oxygen-nitrogen relation even when three subjects are in the chamber. Fresh drying tubes containing calcium chloride to remove water vapor and Ascarite to remove carbon dioxide are

the first time a very economical means of demonstrating the principle of the polarimeter, yet providing results sufficiently accurate for a considerable number of useful and practical analyses.

APPLICATIONS. Extensive tables are available in the literature (*31*, *32*, *139*, *168*, *225*, *283*) for the specific rotation



Courtesy, Carl Zeiss

FIGURE 136. GAS INTERFEROMETER

of a large number of substances, as well as tables in a more convenient form for definite analytical applications. In many instances a combination of the specific rotation plus the rotatory dispersion is useful for identification or for special analyses.

While optical rotation is restricted in its applicability, magneto-optic rotation applies to all substances and in all states of aggregation. This Faraday effect, the numerical value of which is expressed as the Verdet constant, is known for thousands of substances. It has found no practical use in analytical chemistry, largely because of experimental and manipulative complications. Then, too, because of its perfectly general and unspecific nature it is not likely to be of greater use in the future unless experimental simplifications are forthcoming. The magneto-optic effect can be multiplied by repeated passage of the light through the sample, which is not true of ordinary optical rotation.

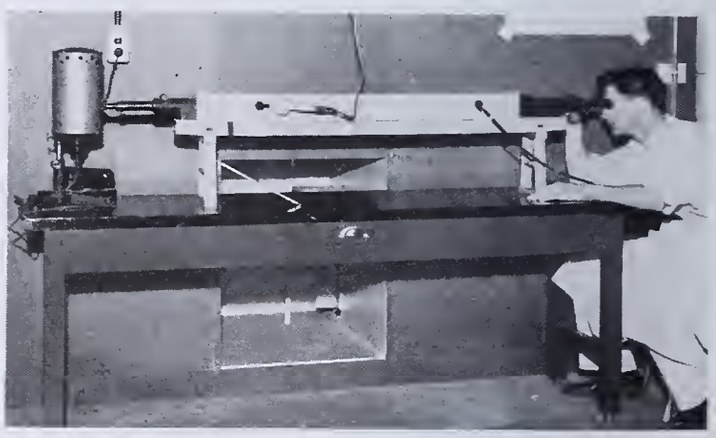
Some interesting possibilities seem to exist in the use of this principle combined with the use of an alternating magnetic field with a photoelectric measurement of the modulated light component (*201*).

Potentiometric

This important branch of analytical chemistry is the subject of several monographs (*131*, *157*, *158*, *159*, *194*) and extensive reviews (*25*, *84*). Böttger's treatise (*25*), which is the most recent and complete, lists 1024 references with titles. Furman's excellent review (*84*) has been brought up to date (1941) and will be published soon. A condensed version was presented recently (*85*) in a symposium on electrochemical methods of analysis. In addition, most modern textbooks of analytical chemistry discuss the subject because potentiometric methods have contributed so extensively to our knowledge of equilibria, solubility, hydrolysis, oxidation-reduction, and the behavior of indicators. For our purpose it will suffice to outline the most important principles and indicate the advantages of this method of analysis.

THEORY. The potential at the electrode-solution interface may be used to obtain information which is analytically useful. For a limited number of electrodes and under suitable conditions, a single measurement of the potential with respect to a known reference electrode gives information which can be used directly. Thus the pH of a solution can be obtained from the hydrogen, quinhydrone, glass, and antimony electrodes. For the most part, changes in e. m. f. during the course of a titration impose less demand upon the reproducibility of the electrode and the number of such systems is much greater. Concentration cells and modifications thereof are also useful.

ELECTRODES. For the determination of pH a number of electrodes are satisfactory. The concept of pH as originally given by Sørensen, $pH = -\log c_{H^+}$, is often written to conform with modern concepts as, $pH = -\log a_{H^+}$. This is not entirely satisfactory, but it is possible to bring the pH scale into closer accord with thermodynamic quantities (*184*).



Courtesy, D. W. Armstrong and P. A. Cole, Division of Industrial Hygiene, U. S. Public Health Service

FIGURE 137. USE OF RAYLEIGH-JEANS INTERFERENCE REFRACTOMETER IN CONTROLLING CONCENTRATIONS OF ORGANIC VAPORS IN AIR

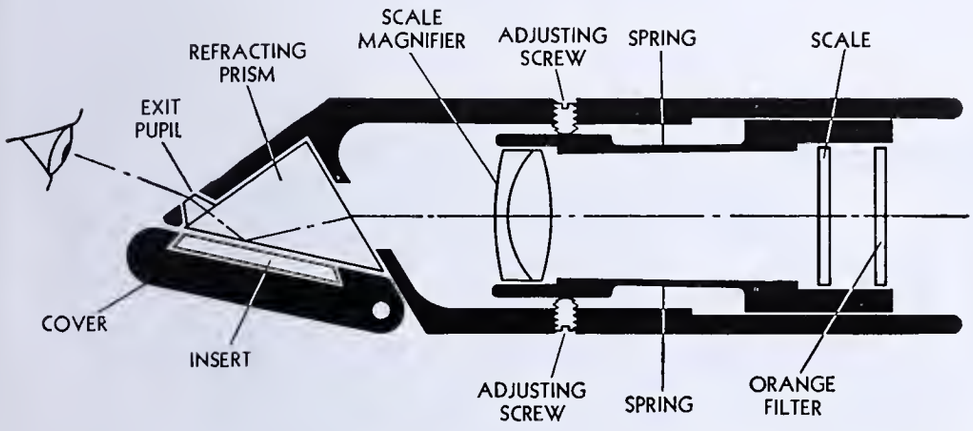
Electrode Systems and Methods. A very great number of electrode systems have been used; these have been classified in several places (*25*, *84*, *158*). Among them are the platinum, glass, antimony, hydrogen, quinhydrone, and silver–silver chloride electrodes, and many bimetallic systems. Exhaustive compilations of individual methods include acid-base (also in nonaqueous media), precipitation, oxidation-reduction, and concentration-cell techniques. These have also been classified (*25*) according to method, substance, class of titration, and application.

Courtesy, Walgreen Co. and Spencer Lens Co.

FIGURE 138. USE OF ABBE REFRACTOMETER TO DETERMINE SOLUBLE SOLIDS (SUGAR) IN JELLIES, SIRUPS, ETC.

Courtesy, Bausch & Lomb Optical Co.

FIGURE 139. HAND REFRACTOMETER



Courtesy, Bausch & Lomb Optical Co.

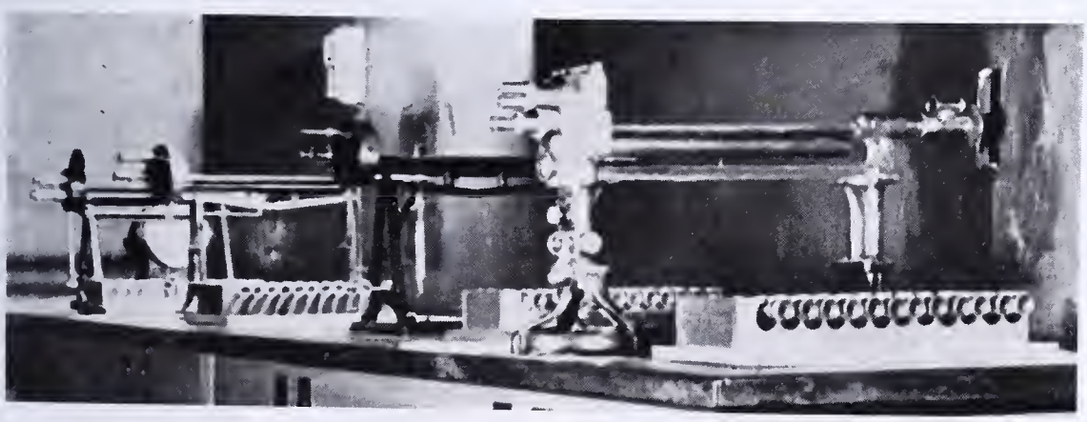
FIGURE 140. DIAGRAM OF HAND REFRACTOMETER

POTENTIOMETRIC TITRATIONS. A typical titration curve is shown in Figure 146, *a*, and in differential form at *b*. Curve *b* is obtained by plotting the change in potential per increment of reagent ($\Delta E/\Delta cc$) against the volume of reagent. Although a titration curve can be computed from the e_0 values, and the value of E at the end point can be predicted, this assumes a high degree of reproducibility of the electrode system and a more or less definite and constant ionic environment. To the extent that these conditions can be met, titration to a definite value of E can be used analytically. In a titration curve, the rate of change of potential passes through a maximum and if this maximum coincides with the stoichiometric point it is merely necessary to locate the maximum; the absolute value of the potential is unimportant. This is the principal problem and its best solution is governed by a number of considerations. Neglecting for a moment the direct instrumental or manipulative methods for doing this, we may note the various end-point criteria in the order of increasing certainty. In the immediate vicinity of the end point the reagent is added dropwise or in small increments of a more dilute reagent and

1. The maximum "throw" or "kick" of the indicating element (galvanometer or meter) is sought. In much routine industrial or control work an off-scale deflection is taken, and anything more time-consuming than this is considered objectionable.
2. A mental note is taken of the maximum throw per drop, thus defining the end point to within a drop or an estimated fraction thereof.
3. Several readings of the potential are taken before and after the end point and the $\Delta E/\Delta cc$ values are tabulated. Simple inspection of the data establishes the end point with some additional opportunity to favor one value a little more than its predecessor or successor (justifiable if the curve is symmetrical).
4. A curve is plotted, preferably with $\Delta E/\Delta cc$ values as ordinates and cc as abscissas (*b*, Figure 146), and the exact location of the maximum is noted.

The general theory of titration curves is discussed in great detail in the texts (particularly well in *25*, *158*). Other mathematical approaches to the location or direct calculation of end points have been made by a number of investigators (*25*, *85*). These depend upon the assignment of an exact or semiempirical equation to the curve and computing the maximum. These methods have been found to predict satisfactory end points from relatively few readings.

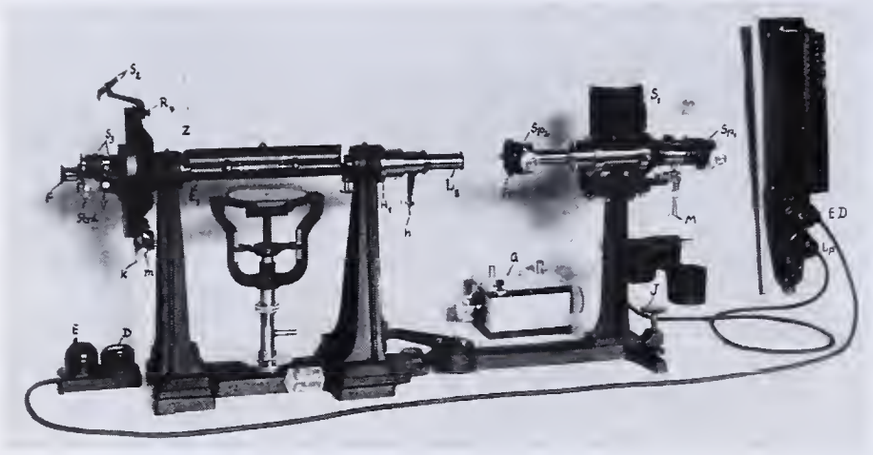
The importance of this problem cannot be overemphasized. Potentiometric titrations are so very useful and capable of



Courtesy, F. W. Zerban, New York Sugar Trade Laboratory

FIGURE 141. SACCHARIMETERS

(*Right to Left*). Bates double quartz wedge saccharimeter (Fric), Schmidt and Haensch double quartz wedge saccharimeters, Bausch & Lomb single quartz wedge saccharimeter. Light sources are behind an insulated wall and laboratory is kept at a constant temperature of 20° C.



Courtesy, Akatos, Inc.

FIGURE 142. SCHMIDT AND HAENSCH PRECISION POLARIMETER ASSEMBLY WITH SPECTROSCOPE-MONOCHROMATOR

The most striking developments are to be found in pH meters, caused largely by the need for acceptable instruments to utilize the useful properties of the glass electrode. [Instruments are manufactured by Beckman (National Technical Laboratories), Cambridge, Cameron, Coleman, Hellige, Leeds & Northrup, and Leitz and by some of these in various models—i. e., research, industrial, or recording types.] The electrode itself has grown apace and is now available in many forms and for specific applications. Examples of one manufacturer's offering are shown in Figures 149 and 150.

There is great need for a definitive treatment of modern potentiometric equipment and two critical reviews have been

Courtesy, National Bureau of Standards

FIGURE 143. CONSTANT-TEMPERATURE BATH WITH CIRCULATING PUMP AND TWO BATES SACCHARIMETERS

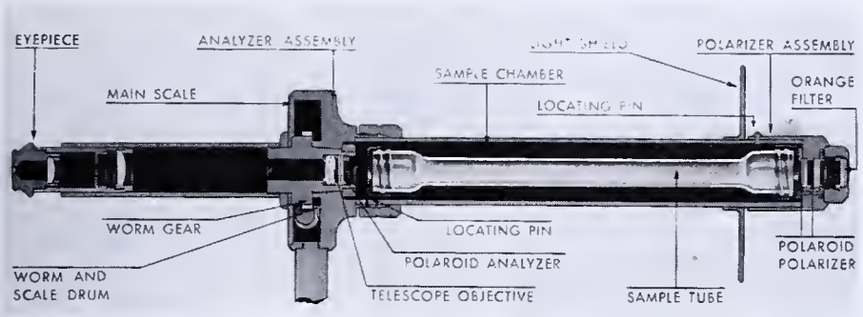
Water from bath is pumped through jackets of polariscope tubes

high precision that any factor which stands in the way of their more extensive use is a challenge. The research man has no aversion to curve plotting or computation—he revels in it. The person called upon to do a large volume of work would like something as striking as the color change of an indicator, yet retaining the unique and distinctive advantages of potentiometric titrations. This seems to be very largely an instrumental problem and criteria in this connection are discussed under that heading.

INSTRUMENTS AND APPARATUS. Individual components of potentiometric equipment have been available for many years. They are offered now in still greater variety and perfection; the participation of the radio industry in this respect is increasingly evident. Aside from equipment intended for instruction, the trend is in the direction of compact units with little or no exposed wiring. A fine example of precision equipment is shown in Figure 147, which combines the reliability of the classical potentiometer with the modern advantages of electronic detection of the condition of balance. A convenient assembly for research is shown in Figure 148.

Courtesy, Spencer Lens Co.

FIGURE 144. POLARIMETER UTILIZING POLAROID FILM



Courtesy, Spencer Lens Co.

FIGURE 145. SECTIONAL VIEW OF SPENCER POLARIMETER

promised for the ANALYTICAL EDITION, one on electrode systems and another on electronics. The need arises from the lack of published details of circuits and technical data on performance. There are one or two notable exceptions to this practice or oversight. All too often performance is designated in chemical rather than electrical terms and adjectively rather than numerically. It may be contended that the chemist is in general interested solely in the former, but it would seem that the minimal information should include numerical data on stability, reproducibility, sensitivity (actual, not engraved scale divisions), input resistance, battery supply voltage effects, or line-voltage variation effects.

Much of the earlier prejudice against vacuum tube devices has disappeared and under the best possible circumstances—demonstrated reliability. The use of the inverted triode or tetrode (*36*) was an important step and the extensive use of the inverse feed-back principle (*222, 272*) has opened up great possibilities for two reasons. This principle reduces the influence of tube characteristics and supply voltage variations to negligible proportions and carries with it the means of devising self-compensating circuits—that is, potentiometric balancing without moving parts. These advantages are still more apparent in automatic recording or control installations.

The principle of differential titration in its various forms involves the use of two electrodes of the same type and some mechanical means for withholding a small portion of the solution around one of the electrodes while the potential is being measured. For each addition of the reagent, small differences in potential will appear until the end point is approached, beyond which they will again diminish. Thus $\Delta E/\Delta cc$ is measured directly. This method has been brought to a high degree of perfection by MacInnes and associates (*185*). Acid solutions can be determined with a precision of 0.003 per cent. Other arrangements have been classified in Furman's review (*84*). The electrical equivalent of this principle has been demonstrated and applied to various classes of titration, using a pulse amplifier (*11*). The sole requirement was shown to be a sufficiently rapid attainment of equilibrium at the electrodes. Shenk and Fenwick (*251*) have discussed automatic titrations.

APPLICATIONS. Potentiometric methods have been applied in aqueous and nonaqueous systems and to organic and inorganic substances and most of these methods have been extended to microtechniques. The summaries by Furman (*84*) and Böttger (*25*) list all applications according to substances and class of titration. Potentiometric methods are characterized by high precision and freedom from many of the difficulties which beset the use of indicators (*136*). The question of speed depends largely upon the system—that is, the ease with which the end point can be detected. Some improvement in this factor can be expected in the future, not in the sense of justifying the use of the methods, but rather in making their extremely wide range of applicability more general.

A few typical industrial applications are included here, Figure 151 showing some measurements of pH in nonaqueous solutions. The extension of these principles to automatic recording and control is in an advanced state of development, and the engineering aspects of this technique are a

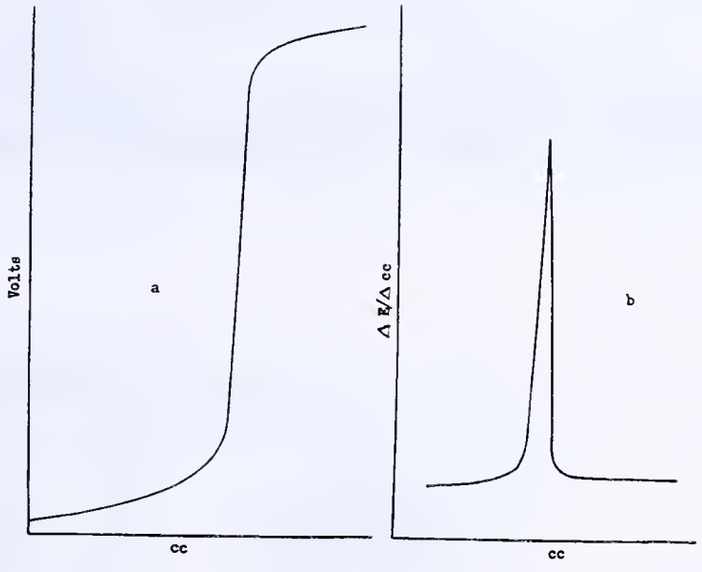


FIGURE 146. TYPICAL TITRATION CURVE

a, direct form; *b*, differential form

Courtesy, Leeds & Northrup Co.

FIGURE 147. PRECISE pH SETUP

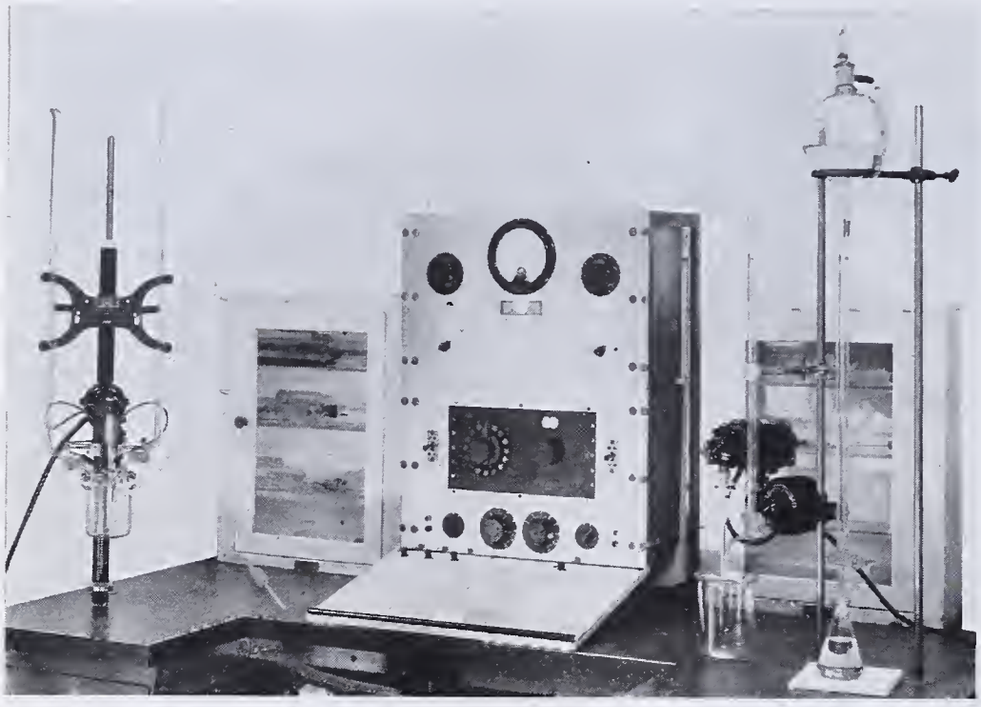
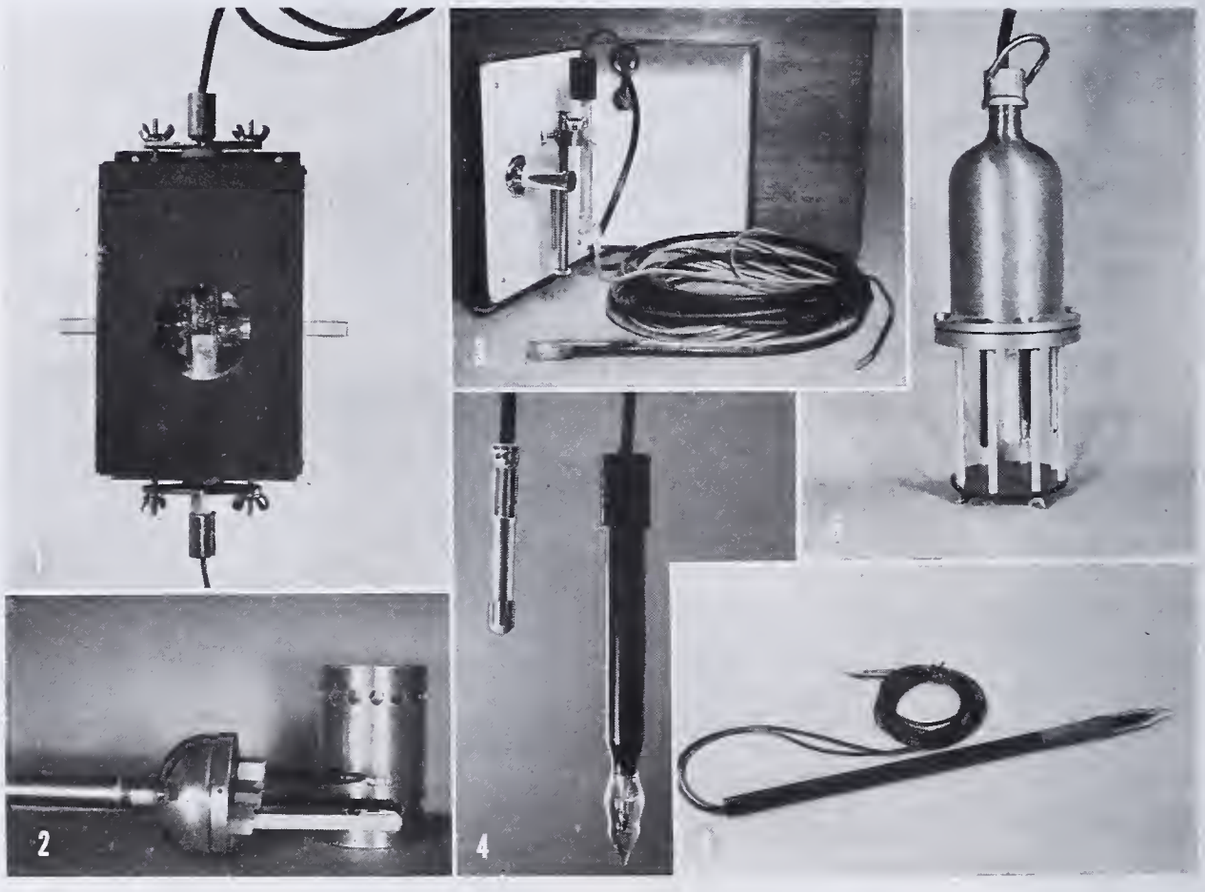


FIGURE 148. POTENTIOMETRIC-TITRATION BAY

Three separate units are available: a conventional potentiometer with illuminated lamp and scale galvanometer, electronic titrimeter (upper section), and electronic section used as vacuum tube galvanometer for standard potentiometer, if absolute values of e. m. f are required. Circuit combinations are easily selected by telephone-type switches.

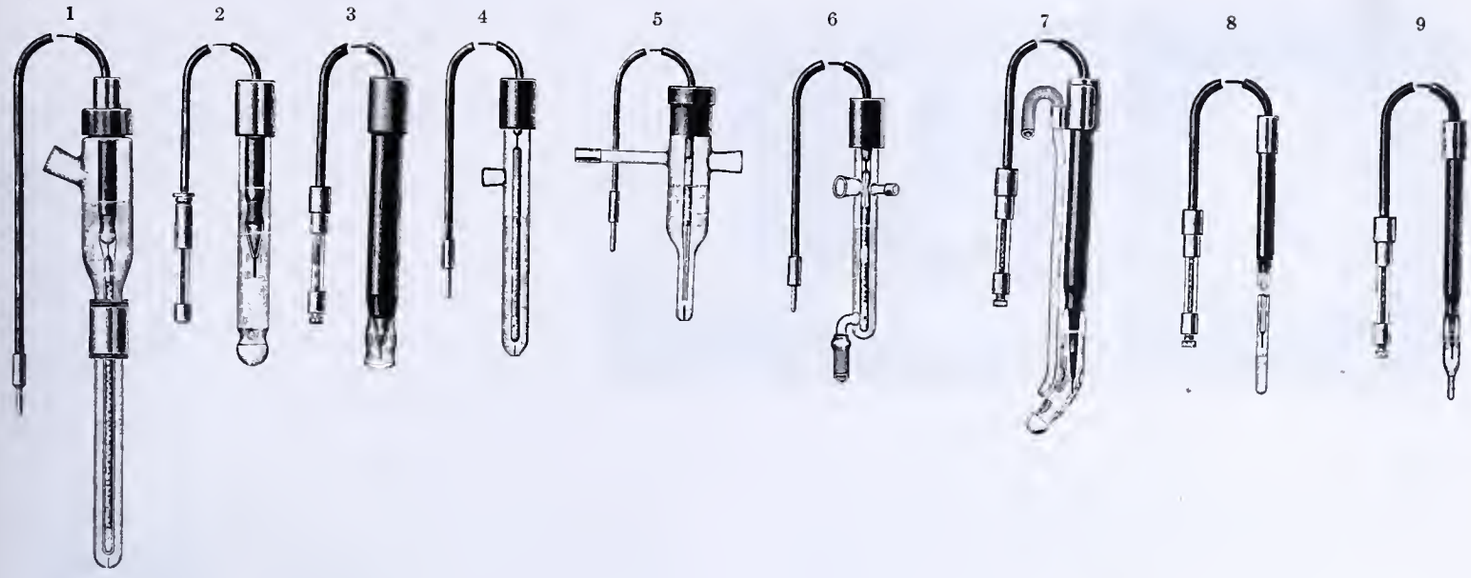
Courtesy, New York University



Courtesy, National Technical Laboratories

FIGURE 149. GLASS ELECTRODES

1. Flow-type electrode assembly for low flows, 50 ml. per minute. Glass and calomel electrodes mounted in Pyrex cross embedded in plaster of Paris
2. Immersion assembly with metal shield, for use in tanks and vats on solutions containing solid materials. All stainless steel
3. Glass-calomel electrode assembly for stomach pH studies *in vivo*. Glass electrode is protected by perforated Lucite head. One small rubber tube is filled with potassium chloride solution to form liquid junction, other tube is for withdrawing samples
4. Spear-type glass electrode for cheese, soil, etc. Internally shielded with integrally attached shielded lead
5. Immersion-type assembly for depths of several hundred feet in plankton studies. Glass and calomel electrodes shown with resistance thermometer. Platinum electrode may also be used for O-R studies. Assembly attached to cable
6. Glass-calomel electrode assembly for vaginal pH studies *in vivo*. Ground-glass shoulder is provided above glass electrode bulb for potassium chloride reservoir and liquid junction. Application assembly attached to Bakelite tube



Courtesy, National Technical Laboratories

FIGURE 150. GLASS ELECTRODES

1. With potassium chloride reservoir, for continuous service, 0° to 100° C.
2. For continuous service, 50° to 100° C.
3. Flat membrane type for pH studies on skin and surfaces, internally shielded
4, 5. Fiber-type calomel electrodes, liquid junction through porous fiber sealed into glass ends
6. Calomel electrode, for mounting on pH meter, with potassium chloride–filled tubing connection to application fittings
7. Glass electrode for vaginal pH studies
8. Internally shielded type for cheese, etc.
9. Internally shielded type for small-animal work

subject in themselves (*197*). Two isolated examples are shown in Figure 152 and others in Figures 153 and 154.

Conductometric

THEORY. Analytical applications of conductance are based on (1) empirical conductance-concentration relationship, and (2) change of conductance during a titration.

In most industrial applications, the necessary calibrations are purely empirical and presuppose that the conductance can be related to the concentration of the desired constituent in terms of some other kind of analysis. Even for a single electrolyte no equation is completely satisfactory over the entire concentration range.

DEFINITIONS. Solutions of electrolytes obey Ohm's law and the specific resistance is defined as the resistance in ohms of a 1-cm. cube. The reciprocal of this quantity is the specific conductance, K. The equivalent conductance, Λ, is given by

$$\Lambda = K \times \frac{1000}{c}$$

where c = concentration in equivalents per liter.

The limiting value of Λ as the concentration approaches zero is called the equivalent conductance at infinite dilution, Λ_0, and the law of Kohlrausch (*101*) states that this value is the sum of the individual ionic conductances—i. e., the sum of the anionic and cationic conductances.

Many expressions have been proposed, relating conductance to concentration (*154*). Modern theories of solution indicate that a square root law is required. For extremely dilute solutions, the equation of Onsager (*184*) expresses the equivalent conductance as

$$\Lambda = \Lambda_0 - \left[\frac{0.9834 \times 10^6}{(DT)^{3/2}} w\Lambda_0 + \frac{28.94\ zi}{(DT)^{1/2}\eta}\right] \sqrt{(z^+ + z^-)\,C} \quad (7)$$

Courtesy, Socony-Vacuum Oil Co. and E. Leitz, Inc.

FIGURE 151. TITRATOR WITH pH EQUIPMENT

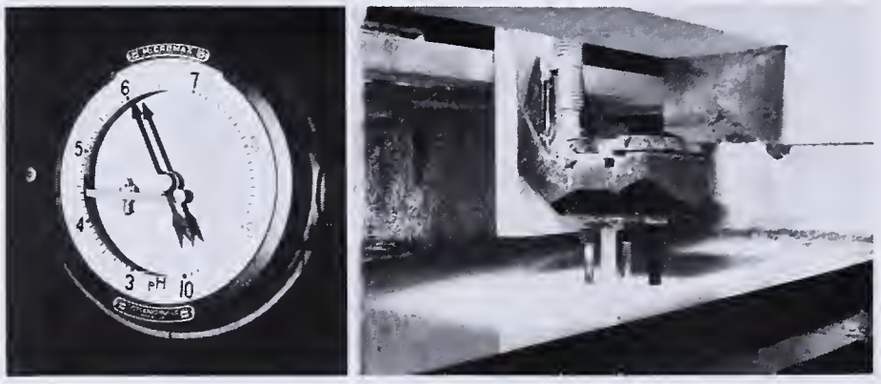


FIGURE 152. pH RECORDERS
Micromax recorder (*left*) and antimony electrode (*right*) detect pH of kaolin suspension
Courtesy, Leeds & Northrup Co.

This holds for any strong electrolyte in any solvent at any temperature and contains no factors which cannot be evaluated. It is extremely important in solution theory but of little practical use for our purpose. Extension to higher concentrations requires the addition of other empirical terms and it is to be regarded as a fundamental limiting law (*184*).

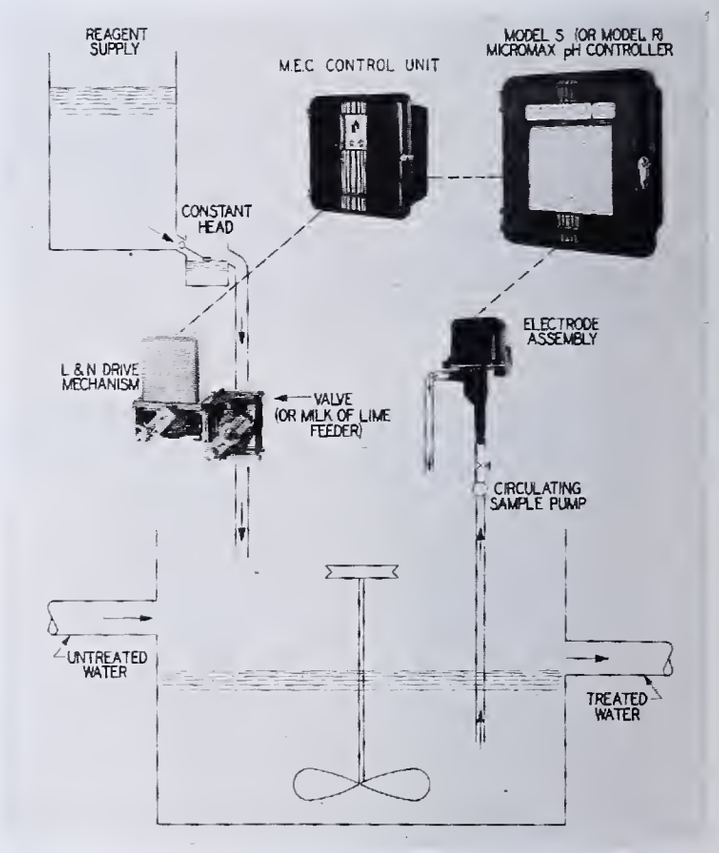
The individual ionic conductances are useful and important in understanding or planning any analytical scheme. Table III (from *184*) gives the values of several of the more important ions at 25° C. The temperature coefficient of conductance depends upon the ion, but amounts roughly to 2 per cent per degree. This is important enough to require temperature control during the measurements, indeed to an extent fixed by the required precision. The conductance of an electrolyte depends enormously on the solvent; indirectly use can be made of this analytically—i. e., in the analysis of mixed solvents.

An important distinction from other electrical methods is to be noted, in that there is nothing characteristic or distinctive about conductance; all ionic species in the system will contribute to the total conductance. For all but the simplest systems, therefore, a titration or selective reaction with one or more kinds of ions must be employed.

Conductance titrations are based upon a measurement of the change in conductance of the solution when one or more of the ions are precipitated, removed to form slightly ionized substances (water), evolved as a gas, or tied up in the form of a complex ion. This process may be expected to cease when these ions have reacted in stoichiometric amount with the added reagent, and the subsequent addition of reagent will cause a further change in conductance, but in a different manner. An "end point" is never obtained directly in this type of titration; rather, one calculates the point at which stoichiometric equality was reached. The various classes of reactions and the course of their conductance have been discussed by Kolthoff and others (*29*, *155*, *156*), and representative examples are indicated schematically in Figure 155. The end point is obtained by the intersection of the two straight lines (*155*). For measurements of very high precision the intersection may be calculated from the equations of the two straight lines (*26*, *192*).

The method presupposes no appreciable change in volume during the course of the titration; hence, reagent concentrations are from 20 to 50 times those of the solution to be titrated and microburets are used. The need for temperature control calls for a thermostat. European practice inclines to the use of an ice bath or constant-temperature jackets built around the cell, using the vapor of a boiling pure liquid.

INSTRUMENTAL METHODS. Conductance cells are available in great variety. In principle the cell consists of two platinum plates of area and spacing dependent upon the order of magnitude of the expected conductance. The solution is contained in a glass or quartz cell between and around



Courtesy, Leeds & Northrup Co.

FIGURE 153. MICROMAX pH CONTROL IN REACTION TANK, USING ANTIMONY ELECTRODE

the electrodes, which have been made in plunge type and pipet style and in rugged pattern to be screwed into pipe lines. The platinum electrode may be bright, sand-blasted, or lightly platinized (platinum black). The relative merits of each are discussed in standard texts. In general, polarization effects are more common on bare electrodes; attainment of equilibrium is more rapid at platinized electrodes; but there are also chances for adsorption or subsequent desorption which may be troublesome in very dilute solutions. Calibration is effected by measuring accurately standardized solutions; the absolute conductance of potassium chloride solutions is known with a high degree of precision (*147*). The cell constant obtainable for such measurements is rarely of any use in titrations, but necessary in empirical conductance measurements.

The resistance of the electrolyte is usually measured with alternating current to avoid electrical as well as concentration-

polarization effects. However, direct current measurements with special arrangements are equal in precision to the best alternating current results.

A simple application of Ohm's law may be used to measure the resistance of the electrolyte. If a constant alternating current potential is maintained across the cell, the current which flows will be inversely proportional to the resistance, or directly proportional to the conductance. This has some advantage in titration work. It is used in the vacuum tube arrangements of Treadwell (*277*) and Ehrhardt (*68*).

The more usual method is to use a Wheatstone bridge in which the conductance cell forms one of the arms. Since the cell is not a pure resistor but also a capacitator, perfect balance cannot be obtained in the alternating current bridge unless it is balanced for the impedance as well as the resistive components. This is usually achieved by shunting the opposite arm with a small variable capacitor.

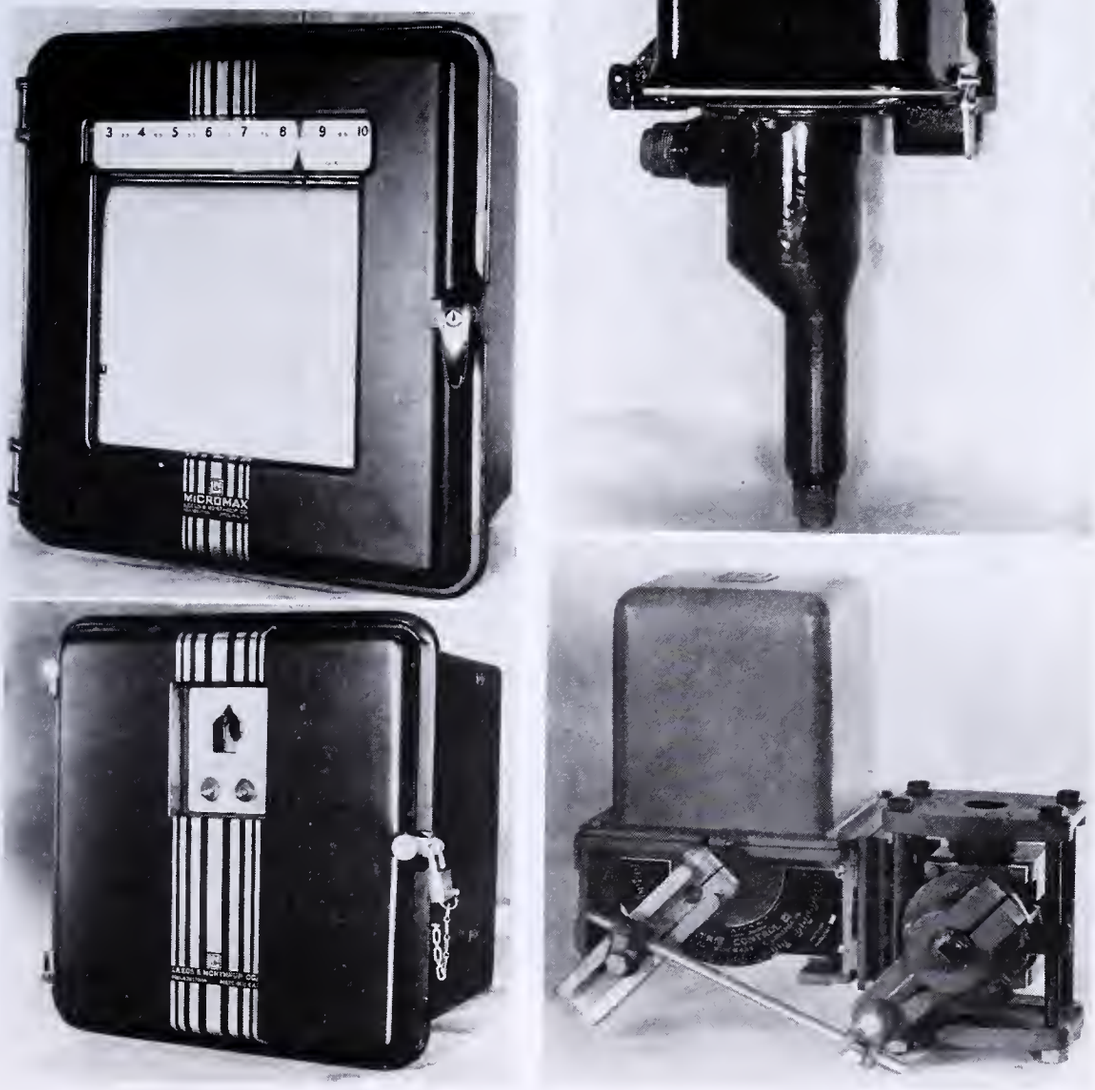
The bridge is fed with a low voltage source of alternating current. Sixty-cycle alternating current is used in commercial conductance bridges, with an alternating current galvanometer to indicate when the bridge is balanced. If a telephone receiver is used, higher frequencies must be employed, and 1000 cycles is a convenient value. In modern work, vacuum tube oscillators are used for this purpose. They are characterized by a wide range of available frequencies and output, good wave form, and cheapness. The telephone null indicator can also be supplemented to advantage with an amplifier, preferably with a gain control. The theory of modern conductance bridges has been thoroughly discussed (*148*, *250*). A fine example of "best" practice in conductance measurements is shown in Figure 156. Needless to say, this is far beyond the requirements of analytical work (*56*).

TABLE III. LIMITING ION CONDUCTANCES AT 25° C.

Cation	λ_0^+	Anion	λ_0^-
K^+	73.52	Cl^-	76.34
Na^+	50.11	Br^-	78.4
H^+	349.82	I^-	76.85
Ag^+	61.92	NO_3^-	71.44
Li^+	38.69	HCO_3^-	44.48
NH_4^+	73.4	OH^-	198
Tl^+	74.7	$CH_3CO_2^-$	40.9
$1/2\ Ca^{++}$	59.50	$CH_2ClCO_2^-$	39.7
$1/2\ Ba^{++}$	63.64	$CH_3CH_2CO_2^-$	35.81
$1/2\ Sr^{++}$	59.46	$CH_3(CH_2)_2CO_2^-$	32.59
$1/2\ Mg^{++}$	53.06	ClO_4^-	68.0
$1/3\ La^{+++}$	69.6	$C_4H_5CO_2^-$	32.3
$1/3\ Co(NH_3)_6$	102.3	$1/2\ SO_4^{--}$	79.8
.....	....	$1/3\ Fe(CN)_6^{---}$	101.0
.....	....	$1/4\ Fe(CN)_6^{----}$	110.5

FIGURE 154. pH CONTROL

(*Upper Left*). Micromax pH recorder for use with antimony electrode
(*Lower Left*). Micromax pH control–valve control unit
(*Upper Right*). Industrial type of antimony electrode for automatic pH control
(*Lower Right*). Motor-energized valve for automatic pH control
Courtesy, Leeds & Northrup Co.



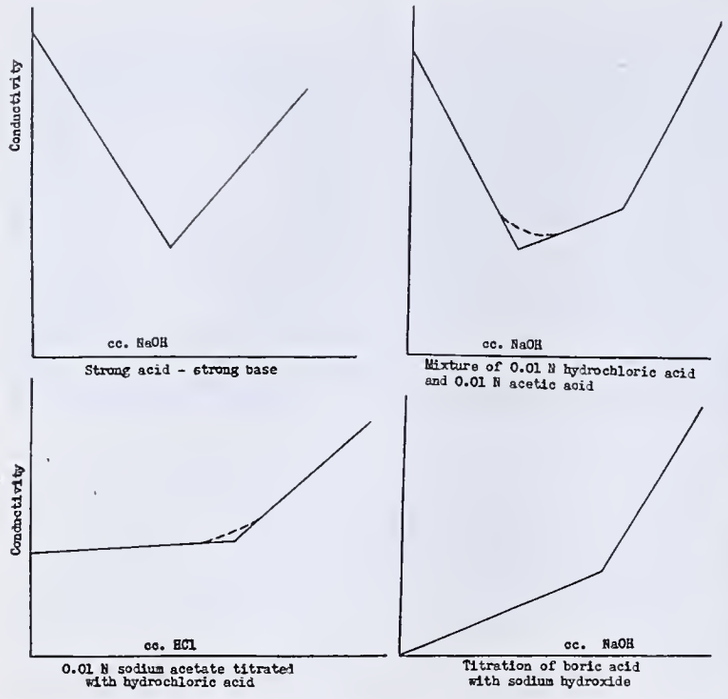

Conductivity
cc. NaOH
Strong acid - strong base
cc. NaOH
Mixture of 0.01 N hydrochloric acid and 0.01 N acetic acid
Conductivity
cc. HCl
0.01 N sodium acetate titrated with hydrochloric acid
cc. NaOH
Titration of boric acid with sodium hydroxide

FIGURE 155. REPRESENTATIVE CONDUCTANCE TITRATION CURVES

The use of telephone receivers is not too convenient nor comfortable. A number of substitutes have been sought and a visual indicator, if sufficiently sensitive, is highly desirable. Some solutions to this problem may be mentioned.

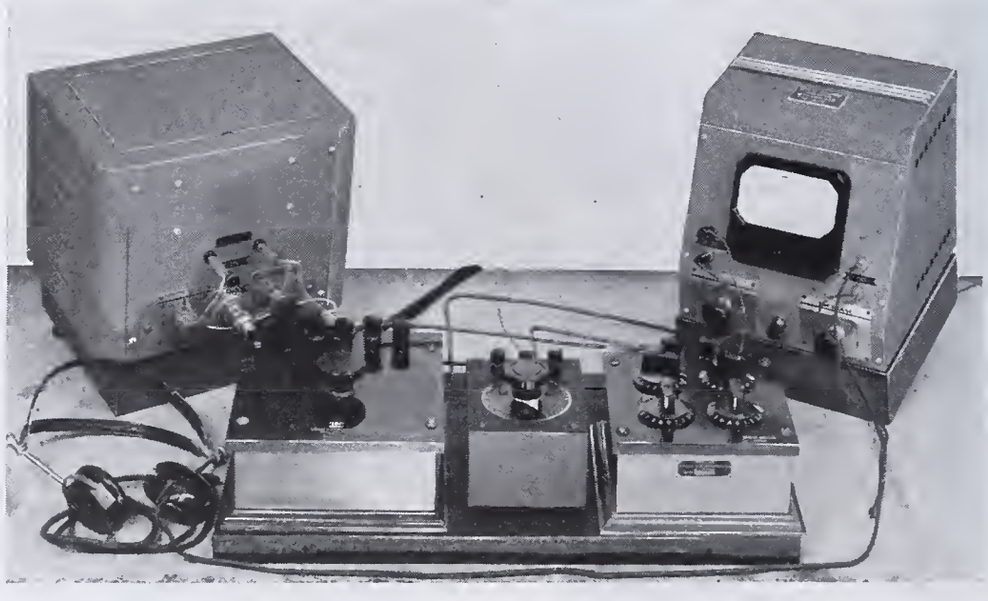
1. Vacuum-thermocouple-galvanometer. The unbalance signal heats a fine wire to which a thermal junction is welded, developing a direct current voltage. The disadvantages are thermal lag and nonlinear response (square law, RI^2).
2. Instrument-type full-wave copper oxide rectifier used with a galvanometer. Response instantaneous (rectifier), nonlinear.
3. Alternating current galvanometers, can be used only at relatively low frequencies.
4. Amplifier-rectifier, direct current meter.
5. Special bridge-balance indicators (electronic) available in great variety. The most ingenious solution seems to be that of Garman (*86*, *87*). With one tube, or in the later model two tubes, a close approximation to the perfect bridge-balance indicator is obtained. The meter deflection is down scale, making damage impossible, and the sensitivity reaches a maximum at zero signal.

Figure 157 illustrates a typical installation for student use.

The compact unit on the left is the vacuum tube oscillator with controllable output, the one on the right is the Garman bridge-balance indicator. The unit in the foreground contains

Courtesy, Leeds & Northrup Co.

FIGURE 156. HIGH-PRECISION CONDUCTANCE ASSEMBLY
Jones bridge, vacuum tube oscillator, output amplifier, Mueller bridge for temperature measurements, and thermostat regulating to ±0.001° C.



Courtesy, New York University

FIGURE 157. STUDENT APPARATUS FOR CONDUCTANCE TITRATIONS, ELECTRONIC SOURCE, AND BRIDGE-BALANCE INDICATOR

a decade resistance box, Kohlrausch slide wire, and Wagner ground. The wiring in the bridge circuit is all exposed; the student makes these connections. Each bridge assembly is plugged into a wall socket, leads from which pass through shielded pipe to the main laboratory thermostat. Similar "station" outlets at the thermostat enable the student to connect his conductance cell to his own bridge circuit.

A complete installation for research purposes is shown in Figure 158.

It is assembled entirely from standard, interchangeable radio equipment including the relay-rack panels. The lower unit contains the oscillator with a choice of three frequencies, 400, 1000, and 2000 cycles, with controllable output. The midsection contains a ratio-arm box and five decade resistor units (both General Radio Co.). No slide wire is used in this bridge. In addition, this unit houses the compensating condenser and the Wagner-ground network. The upper section contains the Garman bridge-balance indicator. The entire instrument is mounted on the wall at a convenient height for the operator. Shielded cable connects with the conductance cell in an adjacent thyratron-controlled oil thermostat (not shown).

The advantages of the newer methods in conductance technique are emphasized by Jander and Pfundt (*25, 141*). They show that present practice permits the analysis of a substance in the presence of a five hundredfold excess of indifferent electrolytes, and that the recent method for determining as little as 0.04 microgram of arsenic is wholly out of the question with the old-fashioned slide wire–telephone arrangement.

Applications. 1. *Direct Measurement of Conductance.* Applications of this class are summarized by Sandera (*238*) with an extensive bibliography of 274 references. To select a dozen at random and indicate the wide range of applicability: conductance measurements have been found useful in determining nitric acid in plant control (*46*), hardiness of winter wheats (*286*), mineral content of wines (*70*), neutralizers in dairy products (*50*), adulteration in maple sirup (*45*), priming of boiler water and condenser leakage (*282*), purity of water supplies (*101*), carbon dioxide and other gases in botanical investigations (*207*), pan boiling control in the sugar industry (*17*), automatic analysis of sulfur dioxide in industrial gases (*78, 193*), properties of yarns and fabrics (*53*), and condition of pork and bacon (*12*).

Among the well-established applications is sugar ash determination, which has largely replaced the tedious gravimetric procedure. The method has been the subject of many investigations (*180, 312, 313*). Figure 159 illustrates the installation of equipment for this purpose in the laboratory of an eminent authority in the sugar field.

Another application is illustrated in Figure 160, primarily because this measurement requires auxiliary equipment peculiar to the nature of the sample. A U. S. Department of Agriculture bulletin (*284*) contains official data and tables for converting the readings of this instrument into the moisture content of typical grains and cereals. The same instrument may be used with a special electrode for determining the moisture content of lumber (see also *265*).

Conductance Titrations. Jander and Pfundt (*25*, Vol. II) summarize the work that has been done on 96 important substances, listing 207 references. This compilation is in convenient tabular form, including useful comments on other applications of the method, special precautions, possible interferences, and estimates of precision or reliability. In this list, in addition to the titration of the commoner inorganic ions, are to be found methods for the analysis of alkaloids, amino acids, aniline, chlorophenols, dimethylamine, fatty acids, tannins, isovanillin, creatine, lactic acid, naphthol, phenol, pyridine, salicylates, thymol, etc.

In a supplementary listing, devoted mostly to microdeterminations (*25*), Jander and Pfundt indicate the improvements which have resulted from modern techniques. The following examples give the minimal quantities analyzed (in micrograms, 10^{-6} gram), the volume of solution, and the precision obtainable: arsenic 0.04 to 100 γ, 3 to 4 cc., ± 10 per cent; lead 1–2 γ, 3 to 4 cc., ±7 per cent; cadmium 1 γ, 3 to 4 cc., ±10 per cent; copper 1 γ, 3 to 4 cc., ±5 per cent; silver 1 γ, 3 to 4 cc., ±5 to 10 per cent; bismuth 10 γ, 3 to 4 cc., ±7 per cent.

The precision which can be expected in a representative conductance titration may be inferred from Table IV, which compares analyses of solutions of technical ammonium sulfate with the gravimetrically determined values. The conductance titrations were made at room temperature after the addition of alcohol.

Table IV. Conductance Titrations of Technical Ammonium Sulfate Solutions (*215*)

Sample No.	SO_3 Conducto-metric %	SO_3 Gravi-metric %	Sample No.	SO_3 Conducto-metric %	SO_3 Gravi-metric %
31	21.05	21.12	40	24.35	24.35
32	20.44	20.49	41	22.64	22.68
33	21.86	21.90	42	21.40	21.53
34	20.82	20.88	43	22.30	22.36
35	22.06	22.13	44	22.56	22.62
36	21.60	21.64	45	19.70	19.60
37	20.62	20.64	46	22.86	22.90
38	21.26	21.34	47	18.69	18.63
39	22.49	22.47	48	22.45	22.45

Values signify grams of SO_3 per 100 cc.

Courtesy, New York University

Figure 158. Conductance Apparatus with Electronic Source of Alternating Current and Electronic Bridge-Balance Indicator

Courtesy, F. W. Zerban, New York Sugar Trade Laboratory

FIGURE 159. ELECTRICAL CONDUCTIVITY APPARATUS FOR SUGAR ASH DETERMINATIONS IN A CONSTANT-TEMPERATURE ROOM (20° C.)
(*Upper Right*) SUGAR ASH BRIDGE. *Courtesy Leeds & Northrup Co.*

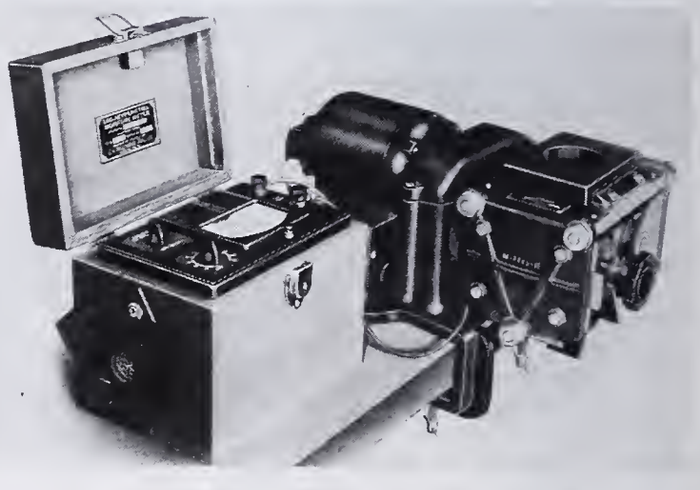
The precision of conductance titrations is generally given as 0.5 to 1 per cent, but this is really no criterion of the technique itself, since conductance measurements can be made to better than 0.01 per cent. To attain this precision would require many refinements in the volumetric technique and excellent temperature control. In addition, titrations involving precipitations are subject to sources of error not found with titrations in which no solid separates (*184*). These errors may arise from coprecipitation, occlusion, adsorption, slowness of formation of the precipitation, or conductance by the suspended solid. In many cases, the errors may be minimized by precipitating in the presence of alcohol.

CONCLUSIONS. Conductance measurements have been applied to a wide variety of analytical problems and there is an extensive literature on the subject. Empirical conductance-concentration relationships have many uses in control analyses and are well suited to automatic recording or control. Conductance titrations are particularly useful in turbid or highly colored solutions or in very dilute solutions. The "end point" is not detected in the ordinary sense of the term, but is found by a graphic or computational treatment of the conductance–reagent volume data. This is, perhaps, the main reason for the relatively unpopular status of this important method. Apparatus of any required precision is available and developments have been very rapid, particularly in the newer electronic instruments. There is no need for more precise measuring equipment. The only possible advantage to be derived from newer instruments is to seek some means for evaluating the end point. The computation of this value is the only bottleneck in an otherwise well-established and esteemed technique.

Electrolytic

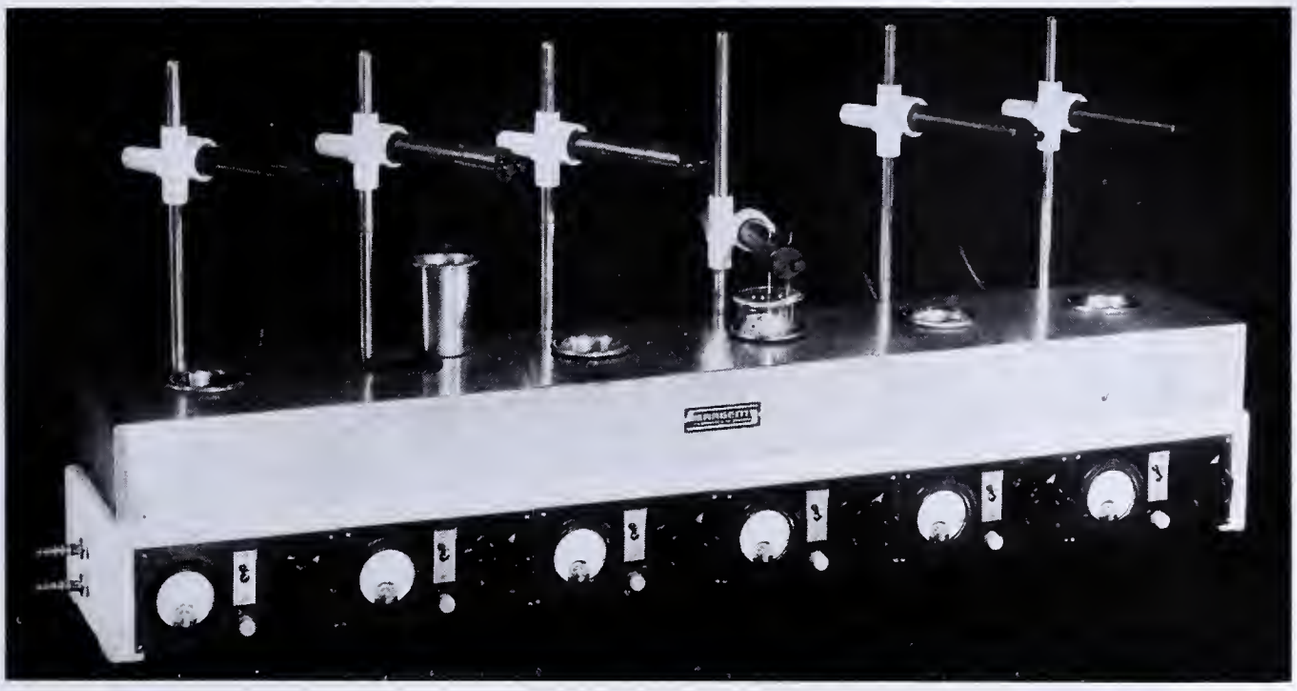
This technique is primarily an adjunct to gravimetric analysis and not an instrumental method in itself, although it seems to have possibilities in this direction. The selective electrodeposition of metals has been practiced for a long time and the foundations of the subject as a distinct means of analysis were largely due to the labors of Classen (*43*) and in this country by Smith (*258*), who was responsible for the introduction of the rotating anode and the mercury cathode (see also *74*). Detailed procedures were developed for a large number of substances and many effective means of electrolytic separation were developed. The method is still widely used, but since its inception the attention of analysts has been directed more to other electrochemical methods such as potentiometric and conductometric titrations, and analysis with the dropping mercury electrode. The subject is discussed here for the purpose of clarifying its relationship to the other techniques.

THEORY. The method is based on the fact that on applying an appropriate difference of potential between two platinum electrodes one can quantitatively deposit metals on one of the electrodes in a form suitable for direct weighing. The character of the deposit is governed by the current density, agitation of the electrolyte, and the possi-



Courtesy, C. J. Tagliabue Co.

FIGURE 160. CONDUCTANCE-TYPE TAG-HEPPENSTALL MOISTURE METER



Courtesy, E. H. Sargent & Co.

FIGURE 161. MODERN HIGH-SPEED MULTIPLE-UNIT ELECTRODEPOSITION APPARATUS, USING ELECTROMAGNETIC STIRRING

bility of codeposition of hydrogen or other metals. The purity of the deposit and its freedom from other metals depend on the relative electrode potentials, and to a large extent the possibilities of codeposition can be avoided by applying their correct potential difference and by a suitable choice of electrolyte. As electrolyte the solution may be acid or alkaline or in the form of complexes such as tartrates, citrates, or cyanides. Most metals are deposited on the cathode, although some, such as lead, are conveniently deposited as the hydrated oxide on the anode. Occasionally a mercury cathode is used on which the metal may be deposited, including the alkalies.

Theoretically the time of deposition can be calculated from Faraday's law, so that as electrolysis is carried out with a definite current the time for complete deposition should be computable. Complications arise which make this impractical, the limitations being due to diffusion effects, a lower rate of deposition toward the end of the process, and a codeposition (hydrogen). Were it not for these complications one might dispense with the final weighing of the deposit and simply calculate the weight of the deposit in terms of the total number of coulombs which were required for complete deposition. The converse process—namely, electrolytic stripping of a deposit under very carefully controlled conditions—seems to have possibilities for a completely automatic electroanalysis involving no weighing (*199*). It is altogether likely that this procedure would have to be limited to relatively simple systems.

Recent developments include the use of microtechniques and the determination of extremely small amounts of metals. The method of internal electrolysis, developed largely by Sand, has many advantages and, as the term implies, requires no applied potential, the latter being supplied by the differences of potential of two suitable electrodes immersed in the electrolyte (*42*).

INSTRUMENTS. Instrumental developments have been mostly in the direction of making these operations more rapid and convenient.

For example, in the instrument shown in Figure 161 the necessity for mechanical stirring of the electrolyte has been avoided by mounting the beaker in a stainless steel vessel which is surrounded by a large solenoid. A strong magnetic field created by the solenoid will cause rapid rotation of the electrolyte when current passes between the electrodes. This is a modernization of the principle developed many years ago by Frary. Provision is also made in this instrument for water cooling and thus appropriate temperature control of the electrolyte. The electrode holders are also greatly improved and provide for automatic alignment with the electrolysis vessel. The electrical controls are all very conveniently mounted for monitoring the applied voltage and electrolyzing current.

Another example of modern equipment is shown in Figure 162, which is entirely alternating current–operated and uses a dry rectifier and filtering circuit to provide direct current for the electrolysis. The necessary meters for current and voltage are provided, and the motor stirrer is very conveniently raised and lowered. These improvements have all assisted in obtaining rapid and accurate analyses. A collection of improved procedures has been published by Slomin (*255*).

Among the micromethods the electrode system of Clarke and Hermance, Figure 163, has contributed greatly to the accuracy and convenience with which small amounts of metals can be determined. One of these, *B*, is particularly suited for the electrolysis of traces of material in very large volumes of solution.

A micro application primarily for qualitative identification consists of a minute Bakelite capsule with two platinum wires molded into the base. If, for example, a trace of copper is sought it may be plated on one of these tiny electrodes and its presence established by microscopic examination, after which it may be removed and subjected to other microchemical tests.

An example of electroanalyses in routine work is shown in Figure 164.

Polarography

This important technique is now twenty years old and is based upon the pioneer work of Jaroslav Heyrovský of Prague. The recent appearance of an excellent monograph on this subject by Kolthoff and Lingane (*161*), the first in the English language, should mark the end of the unaccountable "incubation" period which this subject has undergone in

Courtesy, E. H. Sargent & Co.

FIGURE 162. SLOMIN ELECTROLYTIC ANALYZER

Smooth, stepless adjustment of voltage is made by Variac transformer, making use of rheostats unnecessary. Only a single knob adjustment is necessary to cover entire voltage range.

America. Several recent reviews are also available (*124*, *126*, *160*, *195*), and a complete bibliography (1922 to 1941) of 699 references with titles and author and subject index has been compiled by E. H. Sargent & Company (*239*). These sources provide a complete up-to-date survey in English.

Polarography is based upon the interpretation of current-voltage curves obtained by electrolyzing a solution containing electroreducible or electrooxidizable substances between electrodes, one of which is a dropping mercury electrode and the other a nonpolarizable pool of mercury or any other suitable reference electrode. Under suitable conditions, both qualitative identification and quantitative estimation can be made in one operation. Many organic substances and most inorganic ions can be determined in concentrations ranging from 10^{-6} to 10^{-2} molar. There is no destruction of the sample and it may be used, virtually unchanged, for other studies. Useful information may be obtained with the simplest equipment, but for precise or routine work several elegant instruments are commercially available. These may be manually operated or completely automatic, and with the latter complete "polarograms" may be filed away for future reference.

THEORY. We may discuss the fundamental laws by reference to a typical series of current-voltage curves shown in Figure 165.

The three curves at the right were obtained from solutions containing 0.1 M potassium chloride as supporting electrolyte and cadmium ion in concentrations of 5×10^{-4}, 1.0×10^{-3}, and 2.0×10^{-3} molar, respectively. As the applied voltage is increased no appreciable current flows until about -0.5 volt is reached (the negative sign indicates that the dropping mercury electrode is the cathode). The current then rises rapidly and at higher potentials levels off to a more or less constant value. The height of this plateau above the base line (curve for potassium chloride alone) is called the "wave height" and is a measure of the "diffusion current." The wave height is proportional to the concentration of the electroreducible ion (Cd^{++}). This is shown by the cross-plot on the left, where the increase in current (increment) is plotted against concentration. The vertical dotted line intersects each curve at a point which is equal to one half the wave height. It has a value which is common to all three curves and identifies the ion as cadmium. It is called the half-wave potential and in this case has the value -0.597 volt.

The value for the current at any time, t, during the life of the mercury drop can be expressed quantitatively by an equation derived by Ilcovic (*138*).

$$i_t = 0.732\, nFD^{1/2}Cm^{2/3}t^{1/6} \qquad (8)$$

where i_t = current in amperes at time t
n = charge on the ion (valence)
F = Faraday (96,500 coulombs)
D = diffusion coefficient of ion in sq. cm. per sec.$^{-1}$
C = concentration in moles per cc.
m = weight in grams of mercury flowing from capillary per second
t = dropping time in second

A somewhat more useful form expresses i in microamperes, m in mg. per second, and concentration in millimoles per liter. Introducing the numerical value of F, Equation 8 becomes

$$i_t = 706\, nD^{1/2}Cm^{2/3}t^{1/6} \qquad (9)$$

The rise in current during the life of the drop follows a curve which is a sixth-order parabola. That it truly follows Equation 8 was proved by Ilcovic by using a fast, short-period galvanometer.

It can be shown (*161*) that the average current during the life of the drop is given by

$$i_a = 605\, nD^{1/2}Cm^{2/3}t^{1/6}_{\text{max.}} \qquad (10)$$

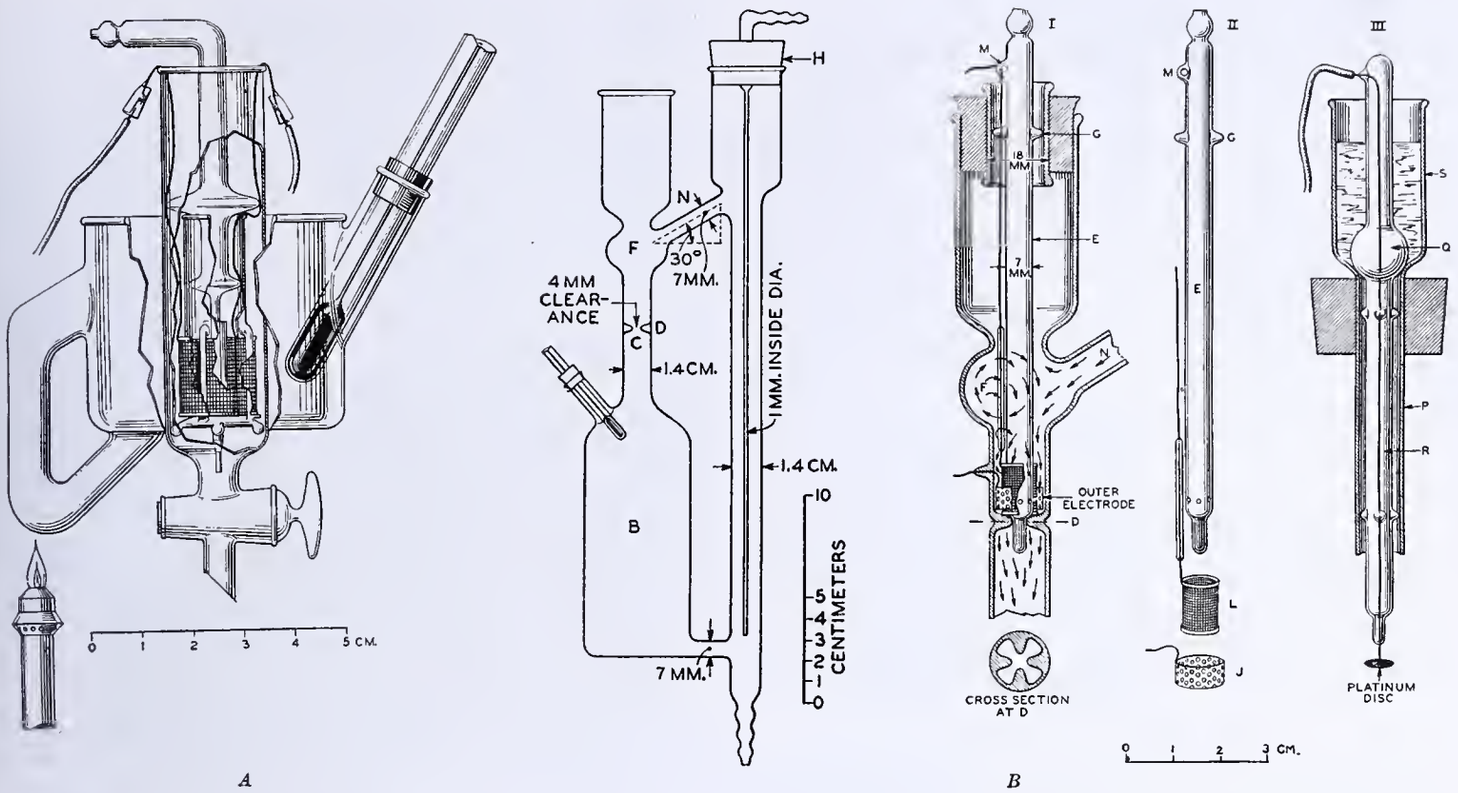
and the maximum current, at the instant the drop falls, by

$$i_{\text{max.}} = 706\, nD^{1/2}Cm^{2/3}t^{1/6} \qquad (11)$$

from which it is seen that

$$i_a = 6/7\, i_{\text{max.}} \qquad (12)$$

It is apparent from Equations 10 and 11 that the maximum current as well as the true average current is proportional to the concentration of the electroreducible or oxidizable substance.



A *B*

Courtesy, B. L. Clarke, Bell Telephone Laboratories

FIGURE 163. MICROELECTROLYTIC CELLS

A. For small volumes
B. For large volumes

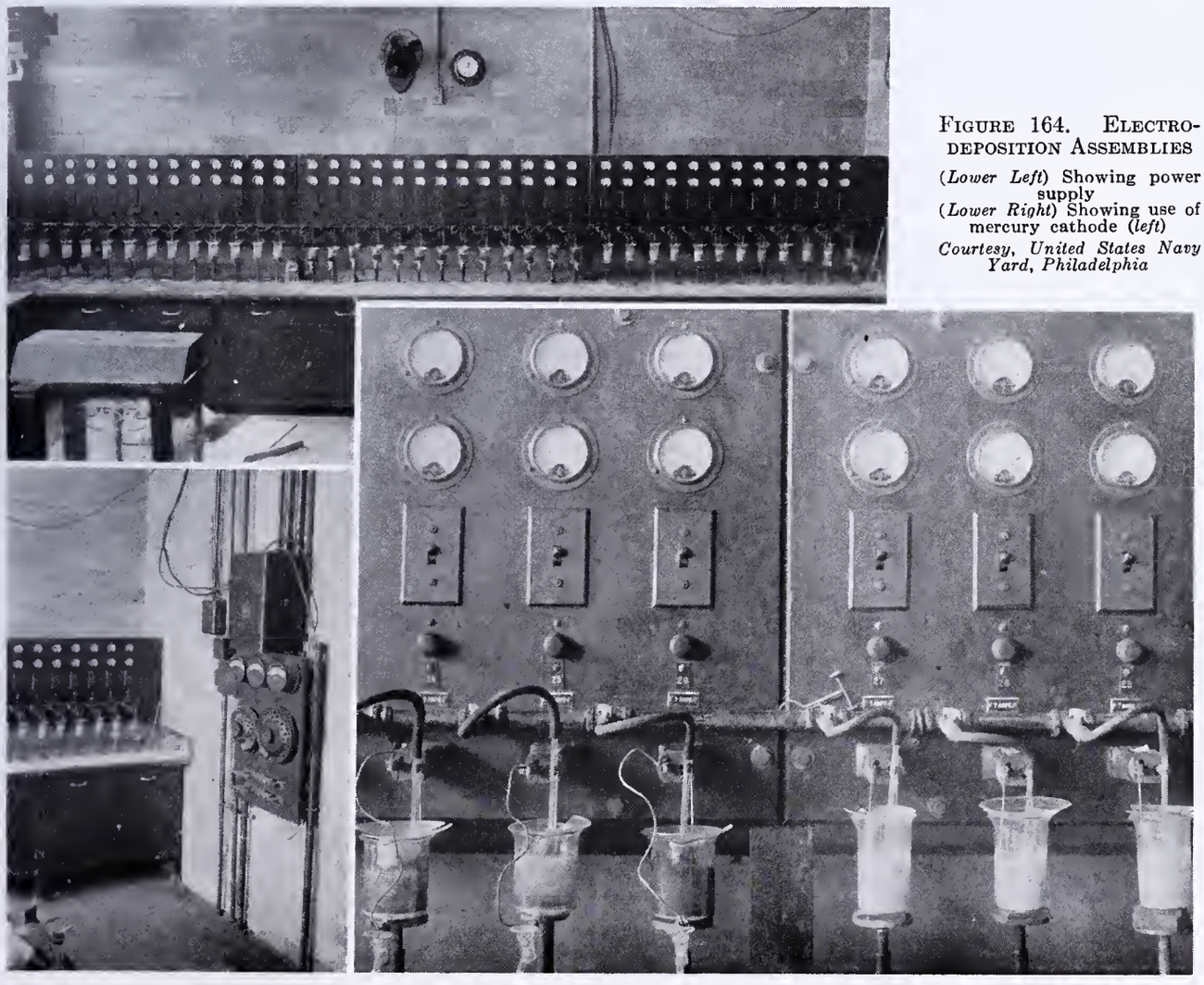
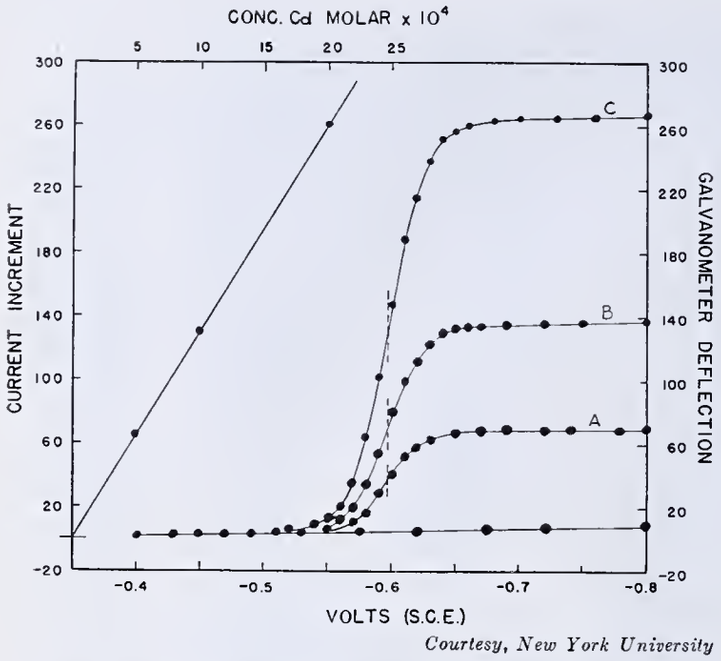


FIGURE 164. ELECTRODEPOSITION ASSEMBLIES

(*Lower Left*) Showing power supply
(*Lower Right*) Showing use of mercury cathode (*left*)

Courtesy, United States Navy Yard, Philadelphia



Courtesy, New York University

FIGURE 165. ANALYSIS WITH DROPPING MERCURY ELECTRODE

These equations have been verified by experiment in all details and are given here to emphasize the factors which must be considered in practice. For satisfactory analytical results, the dropping rate, mass of the mercury drops, and temperature must be controlled. The temperature is involved in these equations in every term except n, including the numerical constant. A complete discussion of this point is given by Kolthoff (*161*), who shows that the over-all temperature coefficient of the diffusion current should be between 1.3 and 1.6 per cent per degree for most of the common ions, and is determined principally by the temperature coefficient of the equivalent conductance of the reducible ion. In practice, a precision of ±1 per cent requires temperature control to at least ±0.5° C. The importance of m and t is evident from the Ilcovic equation. Kolthoff has recommended that all investigators report the value of the product $m^{2/3}t^{1/6}$ in the measurements, and has shown that a knowledge of this quantity enables one to compare results obtained under widely different conditions (*161*, page 61).

The concept of half-wave potential, which defines and identifies the substance, is contained in the equation developed by Heyrovský and Ilcovic (*125*).

$$E = E_{1/2} - \frac{RT}{nF} \log \frac{i}{i_d - i} \qquad (13)$$

where i represents the current for an applied potential E, and i_d the maximum value of the diffusion current at the top of the wave. In Figure 166 the dotted line indicates a current-voltage curve for Cd^{++}. The solid line shows these values recalculated and plotted, with log $i/(i_d - i)$ as ordinates and E values as abscissas. A straight line results, as required by Equation 13, and the value of E for which log $i/(i_d - i)$ is zero is $E_{1/2}$, the half-wave potential. In this case the value is −0.597 volt. The slope of this line should be equal to nF/RT.

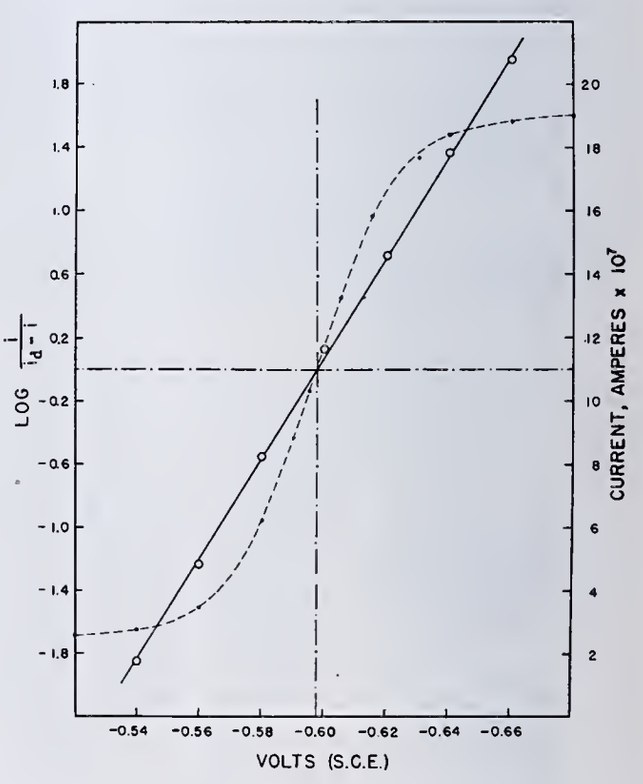
The $E_{1/2}$ value is thus seen to be independent of the concentration and marks the true inflection point of the curve, because the second derivative of E with respect to i, in the above equation, is zero. The use of $E_{1/2}$ has supplanted the older practice of drawing 45° or 35°16′ tangents to the foot of the current voltage curve, because such values are concentration-dependent. However, in some irreversible organic oxidation-reduction systems, no unequivocal value for $E_{1/2}$ can be obtained and these empirical evaluations are used for want of something better.

Under suitable conditions several ions can be identified and determined in the same solution. Thus in Figure 167 is shown a curve for a solution containing copper, bismuth, lead, cadmium, and zinc each at about 0.001 N. The supporting electrolyte in this case was a mixture of tartaric acid and ammonium acetate adjusted to pH 5.1, using bromocresol green and methyl red as a mixed indicator. The indicator dye also serves as a "maximum" suppressor. In this example, the respective half-wave potentials differ sufficiently (>150 millivolts) to permit the wave for each ion to appear distinctly, undisturbed by the others. In general if the $E_{1/2}$ values differ by less than 100 millivolts a satisfactory "separation" is impossible. In many cases, the $E_{1/2}$ values are very close or almost identical. One must then either resort to preliminary chemical separation, or make use of the fact that, since many ions have complex-forming properties, one can change the supporting electrolyte and shift the $E_{1/2}$ values to some more convenient potential. The effect of the medium on the half-wave potential of cadmium is shown in Table V (*161*, page 482).

In some media no reduction takes place at all for certain ions, which is an extreme case of the principle just illustrated.

TABLE V. EFFECT OF MEDIUM

Supporting Electrolyte	$E_{1/2}$ *vs.* Saturated Calomel Electrode
1 N KNO_3, HNO_3, or H_2SO_4	−0.586
1 N KCl or HCl	−0.642
0.1 N KCl or HCl	−0.599
1 N KI	−0.74
0.5 M neutral tartrate solution	−0.80
1 N KCN	−1.18
1 N NH_4OH + 1 N NH_4Cl	−0.75



Courtesy, New York University

FIGURE 166. EXPERIMENTAL VERIFICATION OF EQUATION 13 AND EVALUATION OF $E_{1/2}$

Considering an actual case arising in the analysis of alloys (*135*), it was found that in an ammoniacal solution containing copper, zinc, and nickel a polarogram indicated the copper as a distinct wave, but nickel and zinc appeared together—i. e., their $E_{1/2}$ values did not differ sufficiently—and the curve therefore yielded copper and the sum of the zinc and nickel. By adding an excess of cyanide, in which copper and zinc do not produce a wave, a second polarogram gave nickel alone. (It was necessary to add an excess of sulfite before the cyanide, in order to avoid the formation of cyanate.)

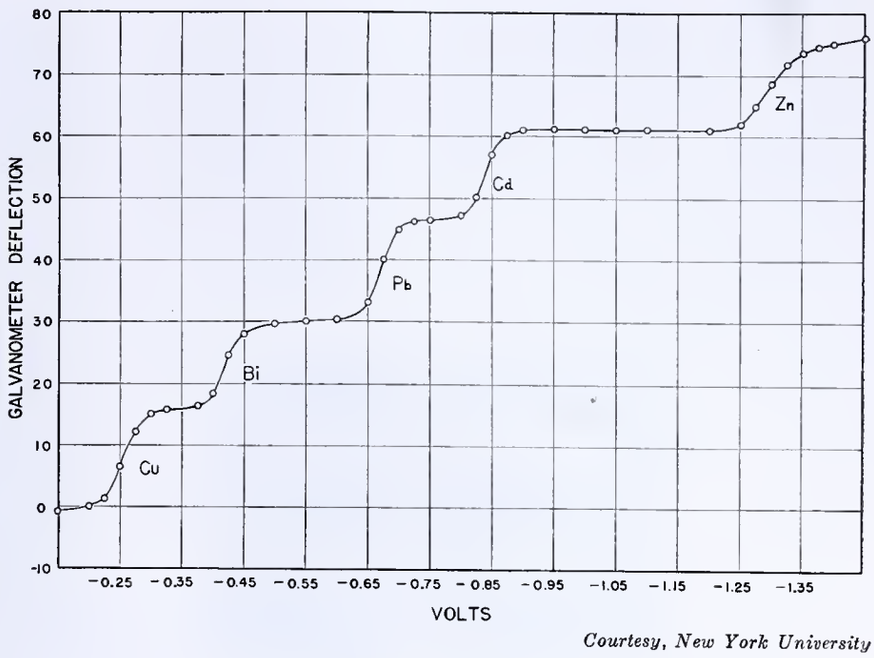
Air must be removed from the solutions, since oxygen is electroreducible at two potentials (-0.1 volt, $O_2 \rightarrow H_2O_2$ and -0.3 volt, $H_2O_2 \rightarrow H_2O$). It is evident that oxygen can be determined at these potentials. Removal of the air may be effected by a stream of pure hydrogen or nitrogen, or in some cases by illuminating gas. Sulfites are often used for this purpose.

In many cases, the current-voltage curve indicates a peculiar type of distortion characterized by a sharp initial rise of the current, which at slightly higher voltages drops back to the normal value. These maxima are highly reproducible. Although they are a nuisance in practical work, they are easily eliminated in most cases by the addition of readily adsorbable dyes, gelatin, glue, etc. Actual use of these maxima has been made in a number of cases, by measuring the progressive decrease in the height of the maximum as increasing amounts of suppressant are added. Thus, minute amounts of starch can be measured by determining the suppression of the maximum obtained in an alkaline cobalt buffer solution. A large amount of work has been done in an effort to elucidate the phenomenon, but many of its aspects are still obscure (*161*).

For the analyst's purpose, all the pertinent, fundamental points have been established and the theory has been checked in all important details by experiment.

Instruments. Satisfactory measurements can be made on a setup assembled from commonly available electrical instruments. A simple voltage divider or potentiometer providing a range of 0 to 3 volts, preferably in three steps, in which differences as little as 5 millivolts can be selected, will serve to apply the potential to the electrode system. A galvanometer is connected in series with the source of potential and the electrodes. A Universal or Ayrton shunt for the galvanometer is necessary because the currents to be measured may range over two or three orders of magnitude (10^{-7} to 10^{-4} ampere). The use of a heavy-duty reference electrode (saturated calomel cell) is becoming increasingly popular, but if the potential is to be measured between the dropping mercury electrode and the stationary pool of mercury, means of switching over to a definite reference electrode is advisable, in order that the anode potential may be checked. The calibration and determination of current sensitivity shunt ratios, etc., follow standard electrical practice (*161*).

Manual. For routine work, a compact, self-contained, and well-designed instrument is to be preferred. Several excellent instruments are commercially available, both in the manual and automatically recording form. A typical example of the manually operated type is shown in Figures 168 and 169 and is based on preliminary development work by the writer (*75*).



Courtesy, New York University

Figure 167. Current Voltage Curve

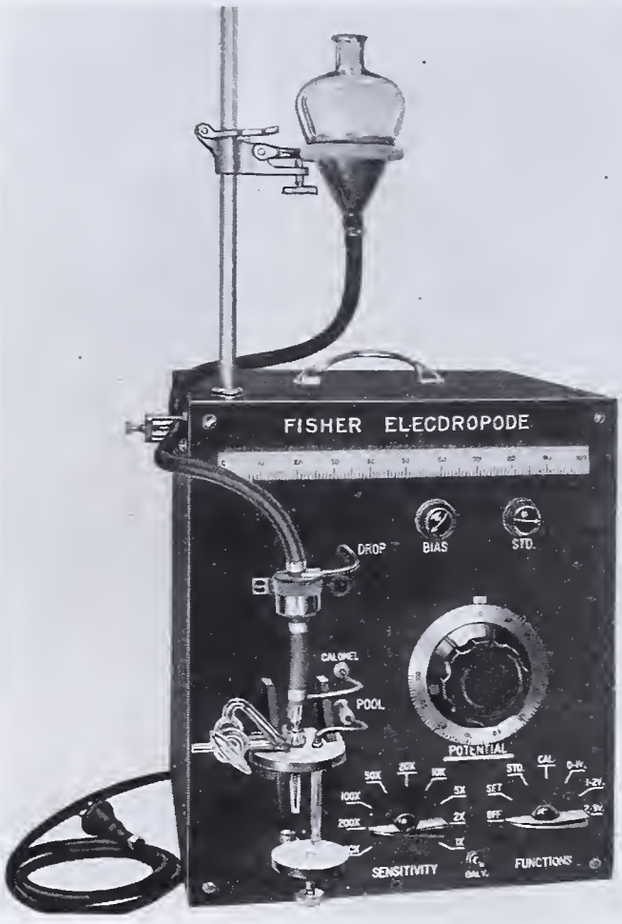
Three potential ranges (0 to 1, 1 to 2, and 2 to 3 volts) may be selected by a switch, and decimal fractions of each range by means of a slide wire. The absolute value of potential is checked in the usual way by balancing potentiometrically against an enclosed standard cell. The galvanometer deflections are magnified by multiple-mirror reflection and appear on the translucent scale at the top of the instrument. Nine ranges of sensitivity are provided by the universal shunt. The polarity of the electrodes can be reversed with a switch which indicates the polarity of the drop. Other controls introduce a bias or compensating current, check the potential of the anode pool, and adjust the mechanical zero of the galvanometer. Considerable merit is to be found in the easily adjusted cell and its provisions for sweeping gas through or over the solution. The curves shown in Figures 165, 166, and 167 were taken with this instrument.

Another example of polarometric analyzer of this class is shown in Figure 170.

In this instrument the setting of the voltage divider is read on a precision voltmeter. Range switches are provided (0 to 1, 1 to 2, and 2 to 3 volts) with coarse and fine adjustment for the working current in the divider circuit and corresponding controls for the applied potential. A sensitive, zero resistance potentiometer-microammeter assembly using a pointer-type galvanometer as a null indicator and the above-mentioned voltmeter are used to measure the current. The sturdy dropping electrode assembly at the left utilizes a large mercury reservoir at the top which utilizes the Mariotte flask principle for constant head and therefore gives a constant dropping rate. This well-constructed instrument is housed in a convenient portable case.

Recording. Heyrovský and Shikata (*127*) invented the polarograph in 1925, for the purpose of relieving the tedium of obtaining current-voltage curves. In principle it consists of a motor-driven voltage divider which is coupled with a recording drum carrying a wide strip of photographic paper. A beam of light is reflected from the galvanometer and focused on the moving paper. The complete current-voltage curve is thus photographically recorded. A complete instrument includes means for standardizing the total voltage impressed across the rotating slide wire, automatic printing of coordinates, and adjustment of the galvanometer sensitivity.

The latest model of the Heyrovský polarograph is shown in Figure 171. The metal housing completely encloses the motor-driven voltage-divider galvanometer and film cartridge. A voltmeter indicates the bridge voltage and a resistor above the meter controls this setting as a vernier to the gross range-setting selector



Courtesy, Fisher Scientific Co.

FIGURE 168. MANUALLY OPERATED INSTRUMENT FOR ANALYSIS BY DROPPING MERCURY ELECTRODE

to the right. The galvanometer deflection is conveniently visible to the operator through the translucent scale at the lower right; and at the upper left a similar port carries the applied potential scale. The galvanometer shunt is located at the lower left and the true current values can be calculated by multiplying the indicated shunt ratios by the galvanometer sensitivity (indicated on the deflector scale in microamperes per division). Additional operative conveniences include a switching arrangement whereby the anode can be connected to the center of the bridge wire, which is very useful for waves which begin at zero voltage, and provision for rotating the camera to new positions when several polarograms are to be recorded on a single sheet. A typical application of this instrument is also shown in Figure 171.

The "Electrochemograph" (Figure 172) is a recording polarograph and consists of a polarizing unit which is a precision voltage divider driven by a synchronous motor, an amplifier, and a standard Micromax recording potentiometer. As the manufacturers point out, the amplifier and recorder are standard units which have wide use for many other purposes in the laboratory. The polarizer unit contains a compensated shunt and a capacitor of such magnitude that the time constant of the circuit is equivalent to a galvanometer of 8-second period. The minute currents are amplified before they are passed on to the recorder and for an output of 1×10^{-6} ampere, the sensitivity to current changes is about 2×10^{-9} ampere. The recorder slide wire is calibrated for -40 to 160 millivolts and the scale is divided into 100 uniform divisions marked 20–0–80. Depending upon the current shunt used, each division corresponds, for the minimum range, to -0.2 to $+0.8$ microampere and for the maximum range to -20 to $+80$ microamperes.

Since the record is a pen and ink trace, the course of the "electrochemogram" is continuously visible to the operator. The recorder chart is also driven by a synchronous motor and is therefore electrically "locked in" with the polarizer unit. One-half inch space on the chart corresponds to 100 millivolts.

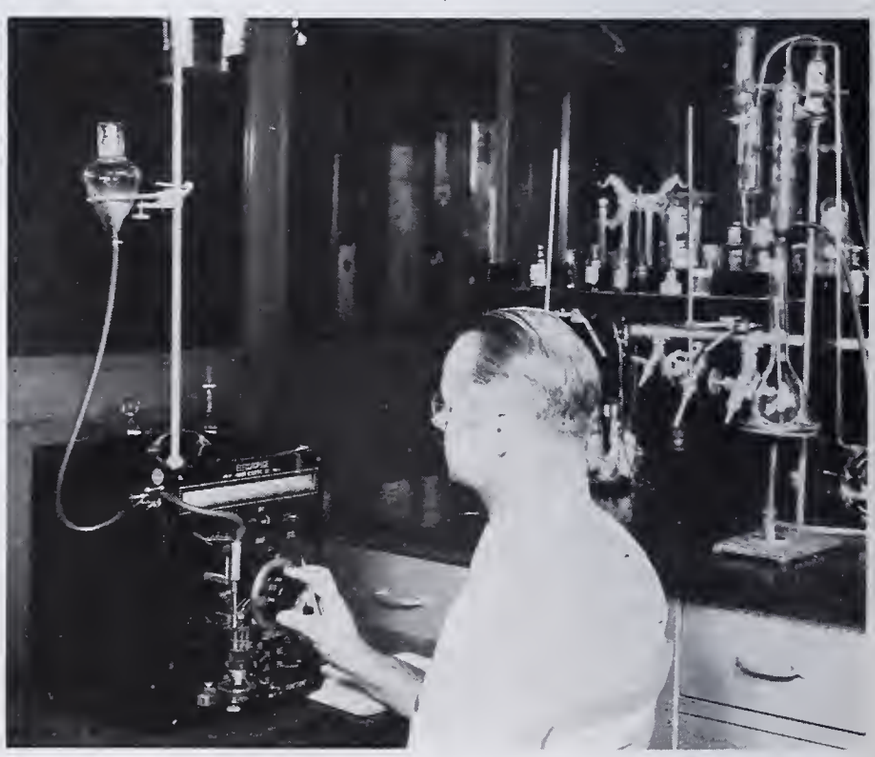
The instrumental approaches to this field have not been exhausted. A few other methods may be mentioned in passing.

The condenser-microammeter method, in which a rugged microammeter replaces the galvanometer and an electrolytic condenser (2000 mfd.) is shunted across its terminals (*161*, page 232). The large condenser practically eliminates oscillations of the needle. For example, with a meter resistance of 4000 ohms, a 2000-mfd. condenser yields an *RC* or time constant of about 8 seconds. Many electrolytic condensers of this class, which are designed for low-voltage filter circuits, may drop to a small fraction of their original capacitance if a potential is not kept across the terminals. In this application no potential of any considerable magnitude is present and this might be expected to occur.

The cathode-ray oscillograph has been applied to the dropping-mercury electrode in three distinct ways, usually not clearly differentiated.

1. By applying a linear voltage sweep synchronized with the drop time (*188*).
2. By applying a small sinusoidal alternating current voltage in series with the direct current potential applied to the dropping electrode. The oscillogram is a sinusoidal wave at a direct current voltage equal to $E_{1/2}$ and is distorted above and below $E_{1/2}$ (*200*).
3. A small sinusoidal alternating current is applied in series with the direct current potential and a phase shift in the region of $E_{1/2}$ is observed on the oscillograph (*24*). In a modification of this scheme, the dropping electrode is connected in a bridge circuit (direct current and series alternating current) and the bridge is balanced for the reactive as well as resistive components. Above and below $E_{1/2}$ the dropping electrode presents a high capacitative reactance. At $E_{1/2}$ the necessary resistance compensation is said to be a measure of the concentration. In the bridge method, no oscillograph is used; instead an amplifier and bridge-balance indicator are used. This is the Dutch equivalent of our "magic eye" (6E5) tube.

APPLICATIONS. Tables of data are given in several places (*124*, *160*, *161*, *195*, *239*) listing half-wave potentials and tangent potentials of organic substances under various condi-



Courtesy, Fisher Scientific Co.

FIGURE 169. USE OF ELECDROPODE IN A LARGE INDUSTRIAL LABORATORY

Courtesy, American Instrument Co.

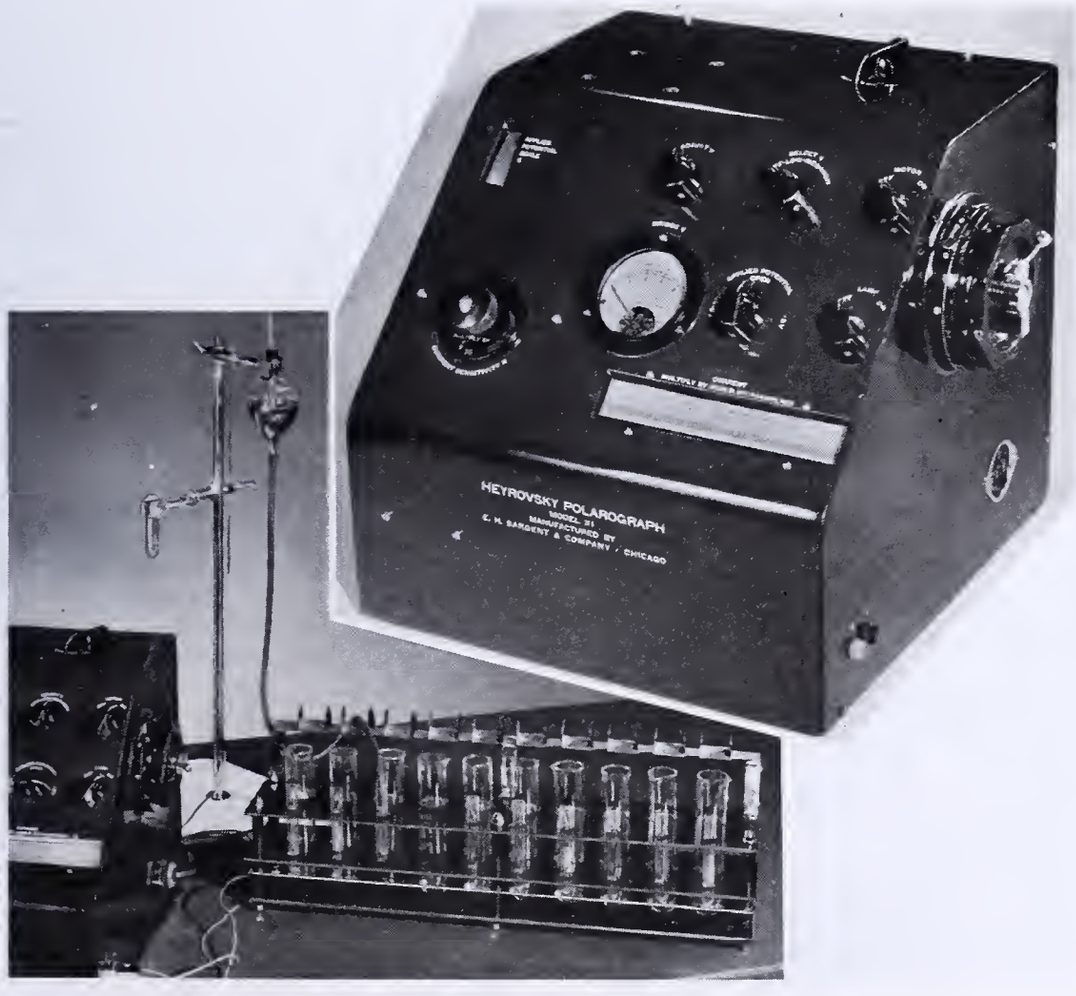
FIGURE 170. POLAROMETRIC ANALYZER

tions and in different media. Actual procedures for technical analyses are also available (*134*) and will suffice in most cases in deciding whether a given determination is feasible. The Sargent bibliography (*239*) is particularly valuable because full titles of all publications to date (1941) are included.

Amperometric Titrations. A most important extension of general polarographic principles is to be found in the method of amperometric titrations (*161*, page 447, *205*). This field is being developed intensively by Kolthoff and his co-workers. Diffusion currents are measured with the dropping-mercury electrode or microplatinum electrode during the progress of a titration. Several cases are recognized in which the substance or the reagent or both are electroreducible. Accordingly, the diffusion current decreases progressively until the end point is reached, or it is zero until excess reagent appears or a V-shaped curve is obtained. The end point is best obtained graphically as in conductance titrations. Corrections are made for dilution effects. There is a degree of selectivity in this technique not to be found in potentiometric titrations, because the applied potential, which is not changed during the titration, can be selected to correspond with the electroreduction of the desired ion. The advantages and limitations are fully discussed by Kolthoff and Lingane (*161*).

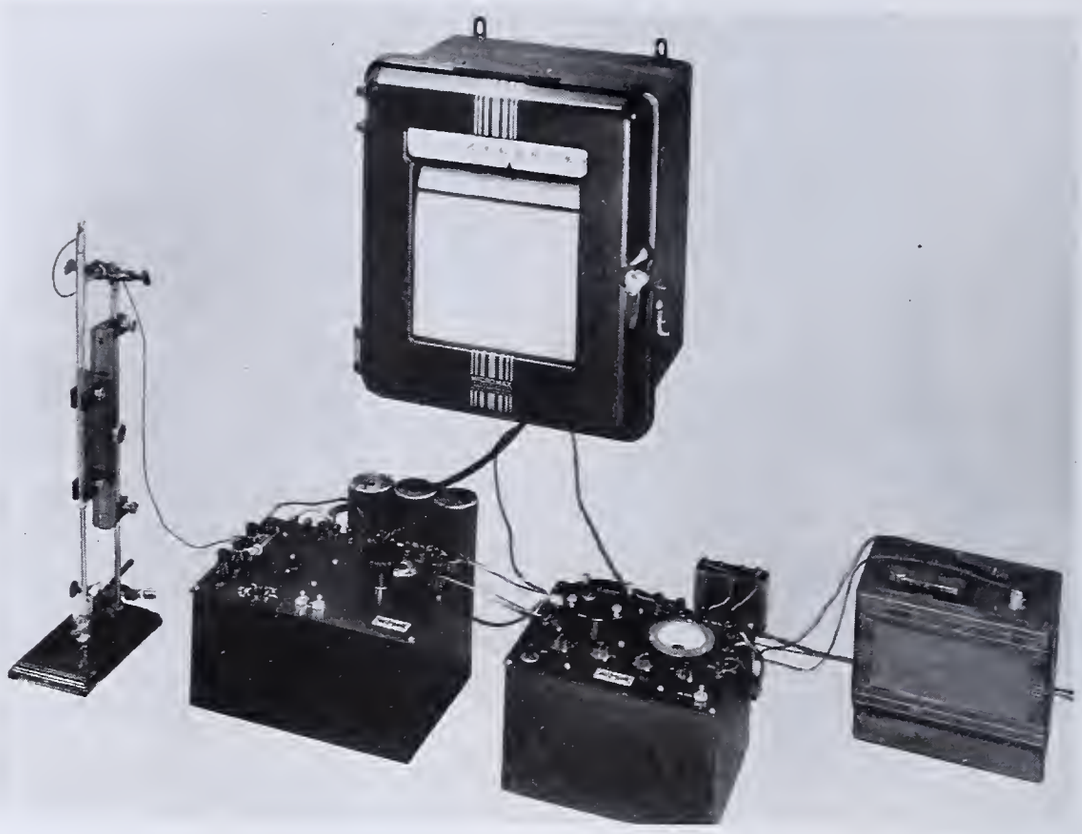
Electrographic

The full resources of Feigl's spot tests (*73*) can be used in unique fashion for cases of analyses which involve the detection of surface impurities and in addition an estimate of their geographic location on the surface. The technique is based on



Courtesy, E. H. Sargent & Co.

FIGURE 171. HEYROVSKÝ POLAROGRAPH AND USE IN DETERMINATION OF LEAD IN URINE
Cells containing prepared specimens are in front row of rack



Courtesy, Leeds & Northrup Co.

FIGURE 172. ELECTROCHEMOGRAPH ASSEMBLY

the early work of Fritz (*82*) and Glazunov (*95*). The technique has been highly developed and improved at the Bell Telephone Laboratories and the following discussion is based largely on the work done there.

Courtesy, Bell Telephone Laboratories

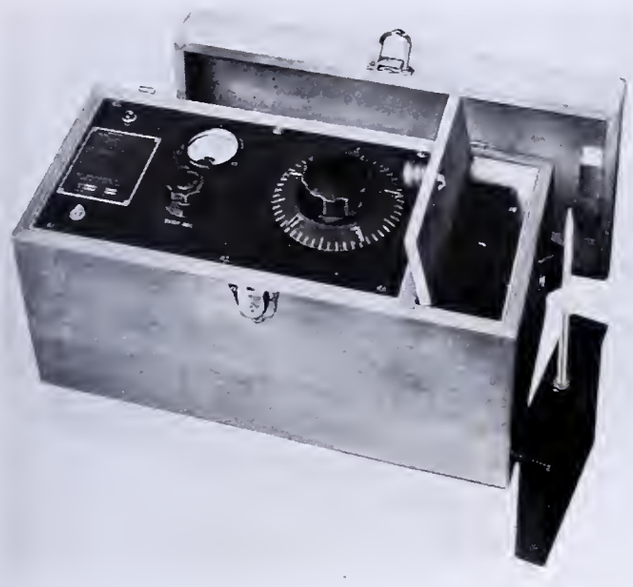
FIGURE 173. APPARATUS FOR ANALYSIS BY ELECTROGRAPHIC METHOD

THEORY. The method depends on the electrolytic deposition of the substances on a reagent-impregnated paper or other suitable matrix. The anode is formed by the test specimen itself and the cathode by some indifferent metal, usually a sheet of aluminum. When a low potential (2 to 6 volts) is impressed, metal ions pass from the specimen into the paper at a rate controlled by the current and in quantity proportional to the time. The deposit produced on the paper may or may not be colored, depending on the choice of reagent, but if it is not it may be suitably "developed" by subsequent treatment of the paper with other reagents. The print is usually sharp because the electric field helps to prevent lateral diffusion. The instrument requirements are fairly simple and the technique is very rapid. The number of available color reactions is very great; indeed, the full resources of Feigl's methods can be utilized.

Very great improvements on some of the original Feigl methods have been made by Clarke and Hermance (*41*)—for example, they have shown that the use of alkali sulfides in test papers is entirely unsatisfactory, since it permits no control of the concentration of that ion. The use of zinc, cadmium, or antimony sulfide provides stable test papers, each of which has its own maximum sulfide-ion concentration and therefore precipitates only those metals whose sulfide solubility products are sufficiently low. The application of these principles to the electrographic technique is an outgrowth of their careful studies and improvements in the spot test technique.

INSTRUMENTS AND APPARATUS. A very convenient arrangement for conducting electrographic tests is shown in Figure 173.

By means of the press illustrated in the foreground an appropriate test paper can be mounted between the specimen and the base plate. The control panel shows meters for measuring the applied potential and the electrolyzing current. Rheostats are provided for adjusting these quantities and an automatic time switch shown above the left-hand meter can be set to control the duration of the "exposure". At an adjacent bench (not shown)



Courtesy, C. J. Tagliabue Co.

FIGURE 174. DIELECTRIC-TYPE MOISTURE METER

complete washing and developing facilities are provided. Small porcelain dishes contain the developing reagents or other media necessary for subsequent development of the image.

Hermance describes this equipment (*119*) as well as completely portable equipment (*120*) for conducting analyses of this type in the field, giving very striking color photographs of technical applications.

APPLICATIONS. A thorough description of the technique has been set forth by Glazunov and Krivohlavy (*96*), who give data for the quantitative determination of nickel in nickel steels and also describe graphic and computational procedures. Jirkovsky (*145*) describes analyses conducted on iron, cobalt, nickel, copper, lead, cadmium, sulfur, bismuth, arsenic, antimony, zinc, and other elements and includes a group of more important references. Hermance's illustrations (*119*, *120*) include studies on tinned brass sheet, which provide striking illustration of porous areas and scratches, indicating exactly where the brass has been exposed, and distinctive prints obtained from nickel-palladium duplex metal rod, and of structural elements which have been plated first with nickel and then with chromium.

In the latter case the print was prepared electrographically with the specimen surface in contact with dimethylglyoxime–barium hydroxide paper. When made anodic, the chromium dissolves directly to form yellow barium chromate, while the nickel reacts with the dimethylglyoxime–barium hydroxide to give a red compound. The distribution of the two metals is therefore revealed in a striking red and yellow print and no ordinary black-and-white photograph can do justice to the sharp and convincing nature of the evidence.

Other examples illustrate the detection of traces of copper or of brass on a penknife blade which gave striking proof of suspected vandalism or sabotage. Although spectrographic evidence has been used in similar cases, it is not difficult to decide which bit of evidence would be more convincing to a jury.

The general technique has been extended by Yagoda (*308*) and others to the location of mineral constituents in plant and tissue sections.

An interesting application of this method has been described by Perley (*214*). In seeking the proper location of recording antimony electrode assemblies it was necessary to find one in which copper was present to an extent no greater than 0.1 part per million, since copper ion interferes with the use of the electrode for pH measurements. A rod of pure antimony was immersed in the solution to be tested, after which the replaced copper was electrographically deposited on reagent paper containing sodium nitrate and diethyldithiocarbamate. The latter produces a brown stain if copper is present. Simple portable apparatus was developed, with which an operator can detect 0.1 to 0.5 p. p. m. of the offending ion.

Dielectric Constant

Dielectric constants can be determined with a high degree of precision and the information in the light of Debye's contributions is very useful in elucidating details of molecular constitution. Analytical applications have been few in number (*61*, *118*); indeed, empirical industrial uses of this method have been more frequent, and the general technique has been applied to control problems—for example, in making continuous measurements of thickness (rubber sheet, etc.). The high state of development of modern instruments would seem to invite further investigation of its analytical possibilities.

THEORY. The dielectric constant, ϵ, of a medium is given by

$$\epsilon = C/C_0$$

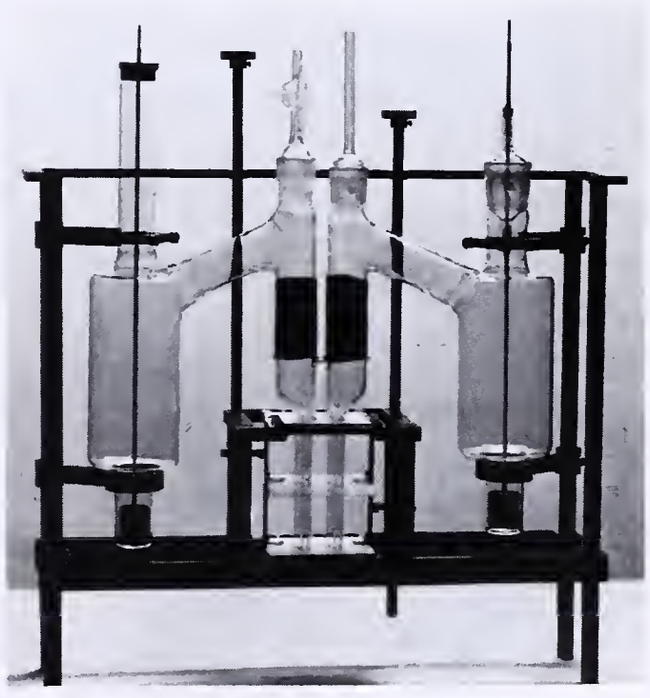
in which C is the capacitance of a condenser with the medium between its plates, and C_0 the capacitance of the condenser with a vacuum between its plates.

For a large number of substances the polarization, P, as defined by the Clausius-Mosotti equation (*44*, *193*) is practically independent of the temperature

$$\frac{\epsilon - 1}{\epsilon + 2} \times \frac{M}{d} = P$$

and from the Maxwell relationship $\epsilon = n^2$, where n is the refractive index for long waves, we would expect identity between P and the molar refraction, and indeed the two agree for many substances, provided P is relatively independent of the temperature.

Debye (*52*) has shown that the polarization contains two



Courtesy, L. G. Longsworth, Rockefeller Institute for Medical Research

FIGURE 175. TISELIUS ELECTROPHORESIS CELL

Courtesy, L. G. Longsworth, Rockefeller Institute for Medical Research

FIGURE 176. COMPLETE APPARATUS FOR ELECTROPHORESIS BY SCHLIEREN SCANNING ARRANGEMENT

Courtesy, L. G. Longsworth, Rockefeller Institute for Medical Research

FIGURE 177. ELECTROPHORETIC PATTERN OF MIXTURE OF PROTEINS IN HUMAN BLOOD PLASMA

contributions, one due to the induced polarization and the other to the permanent dipole moment μ. Thus

$$\frac{\epsilon - 1}{\epsilon + 2} \times \frac{M}{d} = P = \frac{4\pi N}{3}\alpha + \frac{4\pi N}{3} \times \frac{\mu^2}{3kT}$$

It is evident from this expression that the polarization, P, should be a linear function of reciprocal temperature $1/T$ and from the slope of the straight line, one can calculate μ. This equation is the basis for the important contributions which have been made to molecular structure studies.

Courtesy, Burlington Steel Co., Ltd., and Fisher Scientific Co.

FIGURE 178. PLANT USE OF CARBANALYZER FOR DETERMINING CARBON IN STEEL

Some expressions have been derived to predict the dielectric constants of mixtures, involving empirical constants, but in general the dielectric constant reflects all the anomalies of nonideal solutions; indeed, it is one of the most powerful means of investigating deviations from ideal behavior (*261*). This state of affairs does not preclude the use of empirical calibrations for binary or even ternary mixtures, provided the individual ϵ values differ sufficiently. The high value for water (ϵ = 80) makes it possible to measure extremely small amounts of moisture in organic liquids, but by the same token traces of water may complicate the measurement of nonaqueous media.

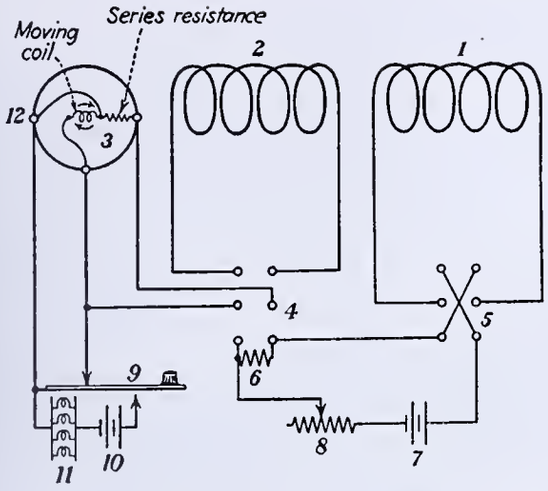
INSTRUMENTS AND METHODS. The dielectric constant is usually measured in terms of the definition which we have given of that quantity—i. e., by measuring the capacitance of a condenser with and without the substance between its plates. Other methods, depending upon resonance, the rate of propagation of electric waves, or a direct measure of the electric force acting through the dielectric are discussed briefly by Smyth (*261*).

Several methods are available for measuring small changes in capacitance. Whatever method is used, it is customary to use the direct substitution, replacement, or compensation technique, in that the measuring condenser is shunted by a high-precision variable condenser. Whatever change occurs in the measuring condenser is cancelled by changing the setting on the precision condenser and since the capacitances of condensers in parallel are additive, the entire burden of precision is placed on the compensator. The rest of the circuit merely indicates when compensation has been effected.

The capacitance bridge is a very convenient instrument for this purpose. Theoretically this consists of a Wheatstone bridge in which the four arms contain pure capacitors and the condition of balance is simple to compute. If a capacitor is "leaky" (finite resistance), the bridge must be balanced for resistance as well as for capacitance, since the currents are out of phase. The balancing of bridges under these conditions has been greatly simplified by oscillograph phase-shift indicators. A very thorough treatment of bridge circuits of all classes is to be found in the monograph by Hague (*103*). The bridge is excited by an oscillator, preferably one with a choice of frequencies. Balance is detected with a crystal detector and galvanometer, or better with one of the electronic indicators (*86*, *87*). Phones can be used if the high-frequency oscillator is slightly modulated at an audible frequency.

In the resonance method an oscillator is used to generate a high frequency. The indicator of this oscillator is coupled to the inductor of the measuring circuit which contains the measuring and compensating condenser. The measuring circuit will have a resonant frequency which is given by

$$P = 1/2\pi\sqrt{LC}$$



Courtesy, H. K. Work, Jones and Laughlin Steel Corp.

FIGURE 179. CIRCUIT OF CARBANALYZER

If the compensating condenser is adjusted until the frequency of the measuring circuit is equal to that of the generator, the current induced in the measuring circuit will be a maximum. The condition of resonance, for which the current is a maximum, can be measured with a vacuum-thermocouple-galvanometer combination or better by electronic means. The resonance curve—i. e., $1/\omega C$—is quite sharp; the true figure of merit is set by the Q of the circuit, which is the ratio of reactance to resistance. Newer developments in radio engineering have furnished increasingly better "high-Q" circuit components.

The heterodyne or beat-frequency method utilizes two oscillators, one of fixed frequency and the other controlled by the measuring condenser. In each oscillator, the frequency is a function of resistance, inductance, and capacitance. In the reference oscillator these are all held constant; in the measuring circuit, the specimen condenser determines the frequency. If the two oscillators are loosely coupled, a new frequency, which is equal to the difference of the two frequencies, appears and can be isolated with a pick-up coil, amplified, and applied to phones or a loud-speaker. By rebalancing the measuring circuit with the compensating condenser, the heterodyne signal can be reduced to zero frequency (zero beat). In practice, this is beset with some difficulties because the oscillators are likely to "pull in" in a sort of lockstep fashion in the region of zero beat. A still more sensitive method consists in setting not to zero beat but rather to some frequency, such as 1000 cycles, and then "beating" this note against a standard 1000-cycle source.

This general technique is practically identical with dozens of contemporary practices in radio engineering, but few of the refinements of the latter field have found their way into dielectric measurements. Frequency measurements can be made with an extraordinary degree of precision (*197*, Figure 14). Certain extensions of the method are discussed below.

APPLICATIONS. Very few applications have been made to analytical problems. Schupp (*246*) in investigating pressed powders and various mixed solids, observed difficulties in the pressed-powders technique due to particle size and the applied pressure. Lampe (*167*) also used the method for moisture in sugar, starch, proteins, and fats and since $\epsilon < 10$ for most of these substances compared with the value for water ($\epsilon = 80$), the value is sensitive to traces of moisture. A recent successful application is illustrated in Figure 174, the dielectric-type moisture meter. The widespread use of this company's conductance-type moisture meter (Figure 160) in official and industrial laboratories has provided ample experience in the demands made upon a practical moisture meter.

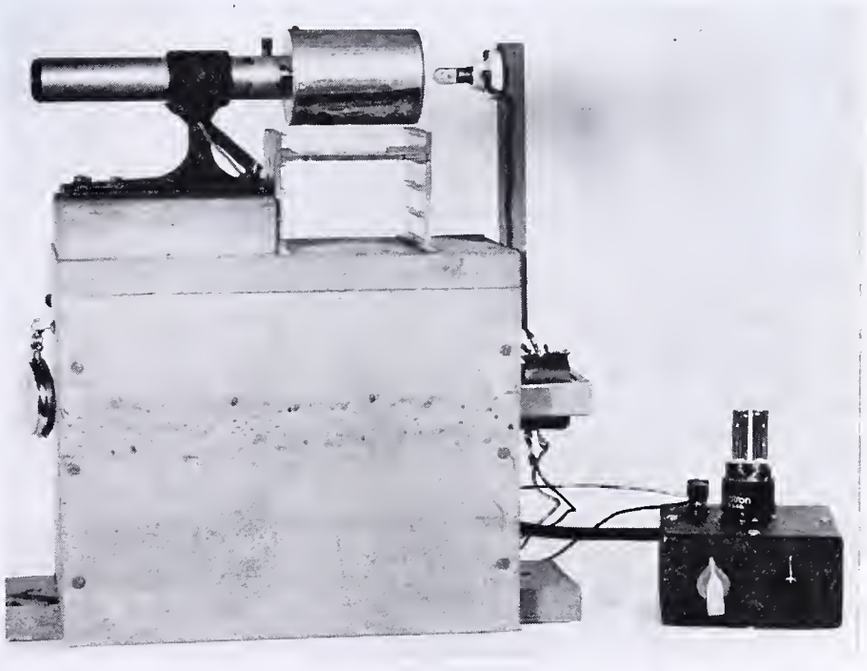
It is intended for use with granular materials such as grains, cereals, and other powdered substances. It is a self-contained alternating current line-operated instrument which uses the heterodyne beat principle. The master oscillator is crystal-controlled and beats against the measuring oscillator, the frequency of which is controlled by the measuring condenser and its parallel compensating condenser. The two frequencies are mixed in part of the master oscillator tube (a pentagrid converter tube), a practice which is common to superheterodyne broadcast receivers. This practice minimizes the tendency of the oscillators to "pull in" and maintains a relatively loose coupling between the two. The difference or beat frequency is rectified by a diode element in one of the tubes and is then fed to a microammeter, which is shunted with a resistor-condenser network to provide a suitable time constant. The measurement consists in adjusting the compensating condenser until the meter reads zero. Small oscillations of the needle will result as this setting is approached, and at the exact setting the motion ceases. Maximum deflections occur at a beat frequency of ca. 200 cycles per second on either side of the true zero setting. The sample condenser consists of two parallel metal plates between which the substance is packed. A satisfactory technique has been worked out for doing this in a sufficiently reproducible manner.

Dielectric measurements are worthy of further study from the analytical viewpoint. The electrical developments are far beyond the chemist's most exacting requirements. There is no scarcity of data, but most of them would have to be recalculated to an analytically useful basis, because the emphasis for the most part has been on questions of theoretical interest, relating to dipole moments and changes in polarization in mixtures as a function of composition.

In addition to the sources mentioned above, reference to treatises on electronics and radio engineering (*222*, *272*) is essential for an appraisal of contemporary practice.

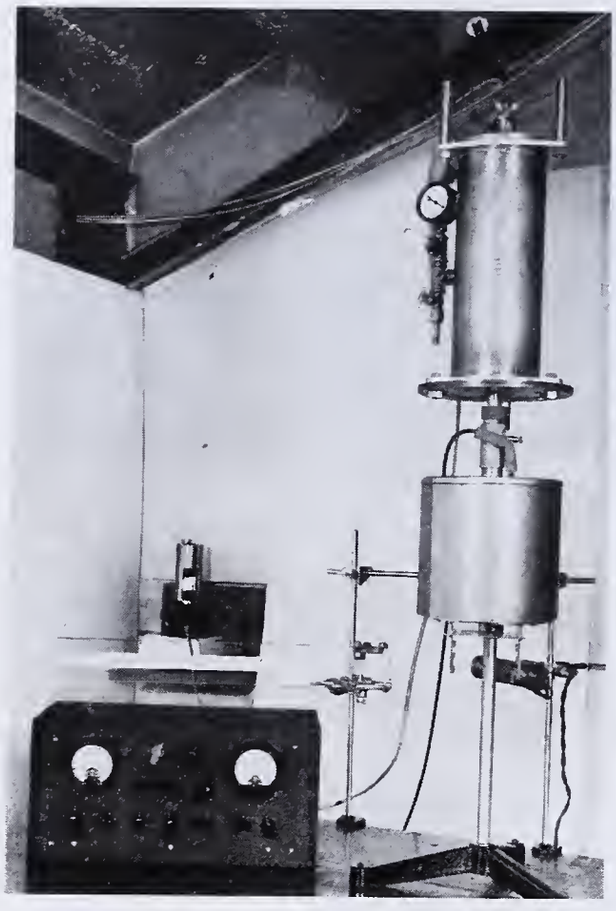
Electrophoretic

Since the phenomenon of electro-osmosis was discovered by Reuss in 1808, the vast field of electrokinetic phenomena has developed. Of the many phenomena and important principles



Courtesy, G. T. Seaborg, University of California

FIGURE 180. LAURITSEN ELECTROSCOPE AND HIGH-VOLTAGE SUPPLY



Courtesy, G. T. Seaborg, University of California

FIGURE 181. IONIZATION CHAMBER AND ELECTROMETER MEASURING CIRCUIT

included in this field (*2*), none is more timely and significant than the study of proteins by the electrophoretic effect. That such studies have succeeded in providing accurate means for analyzing these complex substances is a triumph of first order, comparable only to the alternative and complementary technique of the ultracentrifuge. This interesting and important field was the subject of a recent symposium (*3*) in which some of the leading American investigators participated, and should serve as an excellent introduction to the present status of the subject.

THEORY. In the moving boundary method for analyzing a mixture of proteins, the initially sharp boundary between a solution of the proteins and the buffer solvent will, on passage of an electric current, separate into a number of boundaries, each moving with a velocity characteristic of a component of the mixture. The concentration changes so produced give rise to refractive index gradients, which can be measured in a number of ways.

METHODS AND INSTRUMENTS. Investigations providing precise means for the study of electrophoretic boundaries were initiated by Tiselius (*274*, *275*). At present the methods include the scale method due to Lamm (*166*), the diagonal *schlieren* method of Philpot (*216*) and Svensson (*271*) and the *schlieren* scanning method of Longsworth (*176–178*). The light-absorption method (*3*) is restricted to colored substances or the use of ultraviolet light with prohibitively expensive quartz optics. Since the *schlieren* scanning method yields analytical results with less computational labor than the others, it will be described and illustrated briefly.

Figure 175 shows the electrophoresis cell in which the boundaries are observed, together with the electrode vessels and reversible electrodes. The cell is mounted in a thermostat (Figure 176, center) regulated at 0° C. The gradients of refractive index in the boundaries are photographed by the *schlieren* scanning method. The use of "*schlieren*" or striae for observing such gradients was originated by Töpler (*276*) and a monograph on the technique has been written by Schardin (*240*). Referring again to Figure 176, a light source at the left illuminates a narrow horizontal slit with monochromatic light. The *schlieren* lens is mounted as close as possible to the electrophoresis cell and an image of the slit is formed on the right, at which point a horizontal and movable *schlieren* diaphragm is mounted. The camera lens is focused on the electrophoresis cell and forms a full-sized image in the plane of the camera plate (or ground-glass viewing screen). If the fluid in the cell is homogeneous the image will be uniformly illuminated, but if there is a boundary—for example, between a protein solution and a buffer—there will be a region in which the refractive index varies with the height, and light which would normally pass to the plate is deflected downward, where it is intercepted by the *schlieren* diaphragm. Hence if the *schlieren* diaphragm is raised to a point where it intercepts the most deflected ray, a dark band will appear on the screen, conjugate to the region of steepest gradient in the boundary.

The great advance made by the Longsworth improvement consists in the use of a narrow vertical slit in the plane of the plate and in imparting to the camera plate a uniform horizontal motion. The motion of the plate is coordinated by interchangeable gears with the vertical motion of the *schlieren* diaphragm.

APPLICATIONS. A representative result is shown in Figure 177, which is a photograph of the electrophoretic pattern of a typical mixture of proteins—namely, human blood plasma.

Each peak in the pattern, except that marked δ, corresponds to a component of plasma—namely, *A* albumin, α_1, α_2, β, and γ globulins, and ϕ fibrinogen. The concentration of each component is proportional to the area under the corresponding peak.

The precision of the method is limited only by diffraction phenomena and at present corresponds to about 0.00002 in refractive index—i. e., 0.01 per cent in protein concentration.

Courtesy, R. D. Evans, Massachusetts Institute of Technology

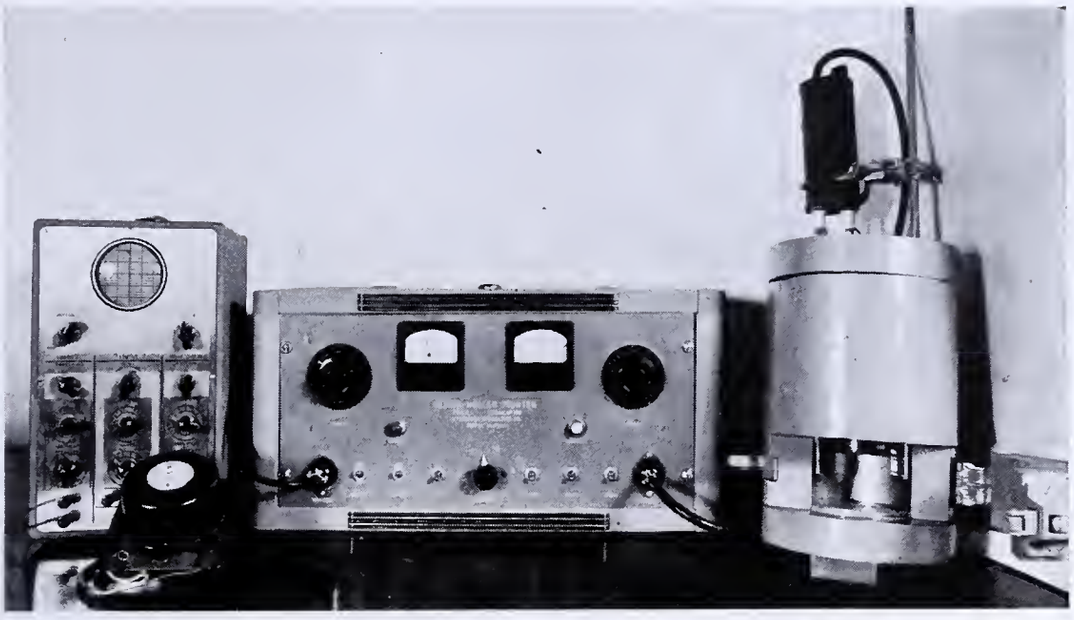
FIGURE 182. GEIGER-MÜLLER COUNTER APPARATUS WITH BELL-TYPE BETA-RAY COUNTER

For a more extensive list of applications the reader is referred particularly to Longsworth (*179*).

Magnetic

The analytical applications of magnetic properties are very limited and few in number. Almost without exception, the measurements are limited to the use of the Gouy balance in conjunction with a powerful magnet. The magnetic susceptibility is a general unspecific property measurable for all states of aggregation and is difficult to measure if high precision is required. It is not surprising, therefore, that practical uses are largely confined to ferromagnetic materials.

The successful correlation between these properties and the carbon content of steels has been mentioned (*197*). The routine examination of a test specimen is shown in Figure 178



Courtesy, G. T. Seaborg, University of California

FIGURE 183. GEIGER-MÜLLER COUNTER ASSEMBLY

and the circuit for the analyzer is shown in Figure 179. The circuit and the general development of this method have been described by Work (*303*).

Radiometric

THEORY. A single radioelement is characteristically defined and identified by its rate of decay, which is given by

$$-dN/dt = \lambda N \qquad (14)$$

or the number of atoms remaining after time t is

$$N_t = N_0 e^{-\lambda t} \qquad (15)$$

where λ = disintegration constant. Another convenient designation is the half-life, T, which is the value of t for which

$$N_t = \frac{N_0}{2}$$

and from which

$$T = \ln 2/\lambda = 0.69/\lambda \qquad (16)$$

If the product of disintegration itself disintegrates, the same type of expression holds and the total activity of the system can be expressed by a simple mathematical extension of the above principle. The various cases for successive transformations are discussed by Rutherford (*235*).

The particles or radiation emitted during disintegration may be detected photographically, by excitation of a fluorescent screen (spinthariscope), or by ionization produced in a gas. In the latter case the process may be rendered visible (C. T. R. Wilson cloud chamber) or measured by appropriate electrical methods.

The unit of intensity in radioactivity is the curie. A curie of any radioactive material undergoes the same number of disintegrations per unit time as 1 gram of radium—i. e., 3.7×10^{10} disintegrations per second (*247*). The type of radiation is classified as follows (*247*):

e^- = negative beta particles
e^+ = positive beta particles (positrons)
γ = gamma rays
e = internal-conversion electrons
K = K-electron capture
$I.T.$ = isomeric transition (transition from upper to lower isomeric state).

Positron emission is always accompanied by "annihilation" gamma radiation, since each positron with an accompanying electron is annihilated and this destruction of mass results in 2 gamma rays each having an energy given by mc^2—i. e., 0.51 Mev. (million electron volts). The previously mentioned methods of detection must be supplemented by further refinements in order to establish with certainty the type or mechanism of disintegration—for example, closed tracks are usually examined in a magnetic field and coincidence counters are used to determine the energy of gamma rays.

Courtesy, R. D. Evans, Massachusetts Institute of Technology

FIGURE 184. USE OF COUNTING RATE METER FOR DETERMINATION OF RADIOACTIVE IODINE IN THYROID OF A PATIENT

ARTIFICIAL RADIOACTIVITY. Before the discovery of artificial radioactivity by Irene Curie and Frédéric Joliot in 1934, analytical applications were limited to the relatively few naturally occurring radioactive isotopes, and although several ingenious schemes were developed to make indirect use of them, the field showed relatively little promise of extensive use. This earlier work is treated by Hahn (*105*) and by Ehrenberg (*66, 67, 210, 211*). Recent tables (*174, 247* (list

more than 350 artificial radioactivities, and radioactive isotopes of 87 elements have been produced (see also *123*, *217*).

They are prepared by bombarding a suitable target by neutrons (n), protons (p), deuterons (d), alpha particles or helium nuclei (α), electrons (e), and gamma rays (γ). Only the first four projectiles are important for preparing useful amounts of the isotopes. The bombardment is carried out in one of the various types of high-voltage apparatus, of which the magnetic resonance accelerator or cyclotron of Lawrence (*116*, *171*) is an outstanding example. Neutron sources using naturally occurring radioactive substances may also be used, but the yields are smaller (*5*). In this type of source, the α particles emitted by radium, radon, or polarium bombard beryllium, which then emits neutrons. Their range or energy may be modified by screens of paraffin or other substances rich in hydrogen atoms.

The production of a radioelement may be represented by an equation such as the following:

$$_{11}Na^{23} + {}_{1}D^{2} \longrightarrow {}_{11}Na^{24} + {}_{1}H^{1} \qquad (17)$$

for the formation of radiosodium by bombarding ordinary sodium with high-speed deuterons, the other product being a proton where the subscript indicates the atomic number and the superscript the mass number of the isotope. The newer and more convenient description is to designate the projectile and the emitted particle; thus the above is a (d, p) reaction. Of the four useful methods, the following types of reactions have been observed and are listed more or less in the order of their probability:

Neutron reactions (n, γ), (n, p), $(n, 2n)$, (n, α), (n, n)
Proton reactions (p, n), (p, γ), (p, α), (p, p)
Deuteron reactions (d, p), (d, n), (d, α), $(d\text{-}2n)$
Alpha particle reactions (α, n), (α, p), (α, d), $(\alpha, 2n)$, (α, α)

This terminology designates the process; the complete statement also specifies the target, so that Equation 17 is abbreviated to Na-*d*-*p*. Thus the four methods for preparing radiosodium may be abbreviated as Na-n-γ, Mg-n-p, Al-n-α, Mg-d-α. In the last case, the effective target is really the stable isotope of magnesium (Mg^{26}), which is present to the extent of 11.1 per cent in ordinary magnesium.

The valuable table by Seaborg (*247*) gives complete information on more than 350 artificial radioelements, listing the atomic number, mass number, degree of certainty of the assignment, type of radiation emitted, half life, energy of the radiation (particle and gamma rays) in Mev., and the various modes of production. Another table in this splendid paper lists the stable isotopes of the elements, giving mass numbers and the relative abundance of the various isotopes. These tables really provide a list of "reagents" for the prospective investigator and he can form in advance a fairly good opinion of their suitablity for a given problem.

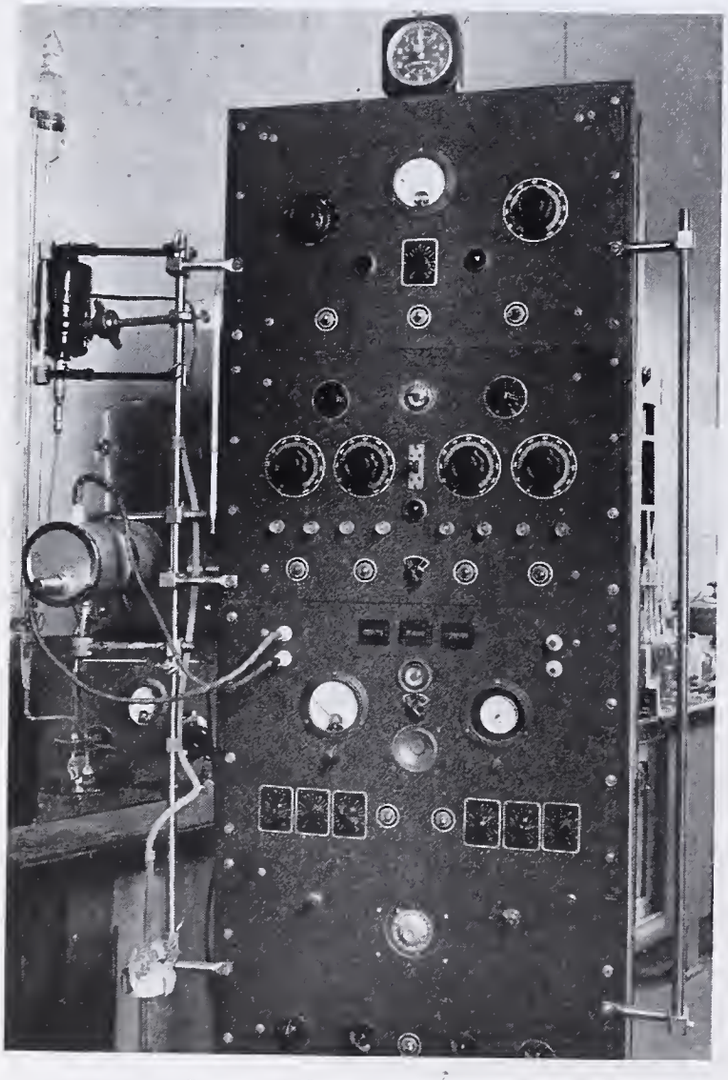
Separation of Radioelements. The preparation of a radioelement is one thing; its isolation or concentration in a form suitable for use is another and requires considerable care and ingenuity. The various methods have been reviewed and classified (*227*, *247*). The techniques may be grouped as follows (*227*):

1. Electrochemical
   - Gas phase
   - Liquid phase
2. Chemical
3. Extraction
   - By solvents
   - By absorbents

If the radioelement which has been formed is not an isotope of the target element, its removal is facilitated by the addition of a small amount of the corresponding inactive isotope ("carrier") and it is then subjected to the usual methods of chemical separation. In general, it is desirable to maintain a high "specific activity"—that is, ratio of active to inactive isotope. There is still ample opportunity to discover new chemical procedures to improve and refine this part of the problem (*76*).

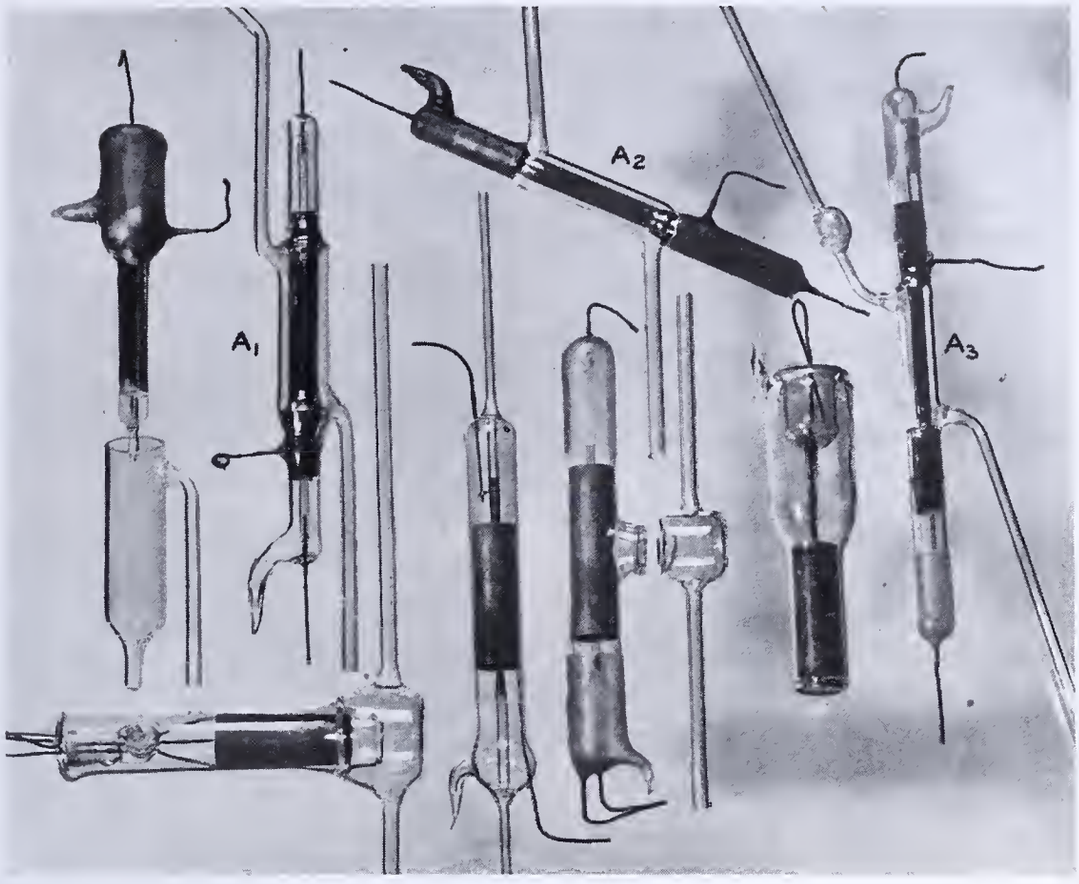
It may be of interest to appraise the "production facilities" of modern devices. Under the best conditions, amounts of the radioelements can be prepared which are just about weighable. Thus, according to Seaborg (*247*), a 4-hour bombardment of phosphorus with 100 microamperes of 16-Mev. deuterons produced about 50 millicuries of P^{32}. From the known disintegration constant this can be shown to correspond to about one-sixth microgram of radioactive P^{32}. Another aspect of the situation may be inferred from the statement of Kurie (*163*) that the demands put on the 37 inch cyclotron at Berkeley are so great that it is operated about 19 hours a day. This places such a great premium on efficient bombardment that the maximum current is employed and pure elements instead of compounds are used. The fact that nearly a kilowatt of energy must be dissipated in the target indicates the enormous energies involved, not to mention many technical and operative difficulties encountered in maintaining an intact target.

Measurement of Radiations. The choice of detector is governed by the type of radiation which is to be measured as



Courtesy, A. Langer, Westinghouse Research Laboratories

Figure 185. Radiometric Titration Arrangement with Counter



Courtesy, A. Langer, Westinghouse Research Laboratories

FIGURE 186. TYPES OF LIQUID COUNTERS

A_1, A_2, A_3. Liquid-jacket-pipet counters used for titration

well as the sensitivity and stability which are required. In general, the measurement is based on determination of the ionization produced by the radiations.

One of the simplest devices is the Lauritsen quartz-fiber electroscope (*170*). The instrument is somewhat similar to a gold-leaf electroscope, but it is much reduced in size and uses a gold-coated quartz fiber in place of a gold leaf. The deflection of the fiber is measured with a low-power microscope provided with a micrometer ocular. The response is linear over 0.6 to 0.7 of the scale range, or over the entire range by offsetting the zero point. With a 3μ quartz fiber the average sensitivity is about 1 division per volt. The capacity of the unit is about 0.2 cm.; therefore, the ion sensitivity is about 1.5 million ions per division. Slightly higher sensitivities may be obtained (700,000 ions per division) by using a lighter fiber. In terms of radioactivity measurements, this instrument works best for intensities of the order of microcuries (10^{-6} curie), although 10^{-7} or 10^{-8} curie can be measured under the best conditions (*247*).

The sensitive element may be placed right in the ionization chamber; indeed, it can be placed in a volume as small as 0.5 cc. and used in thimble chamber measurements.

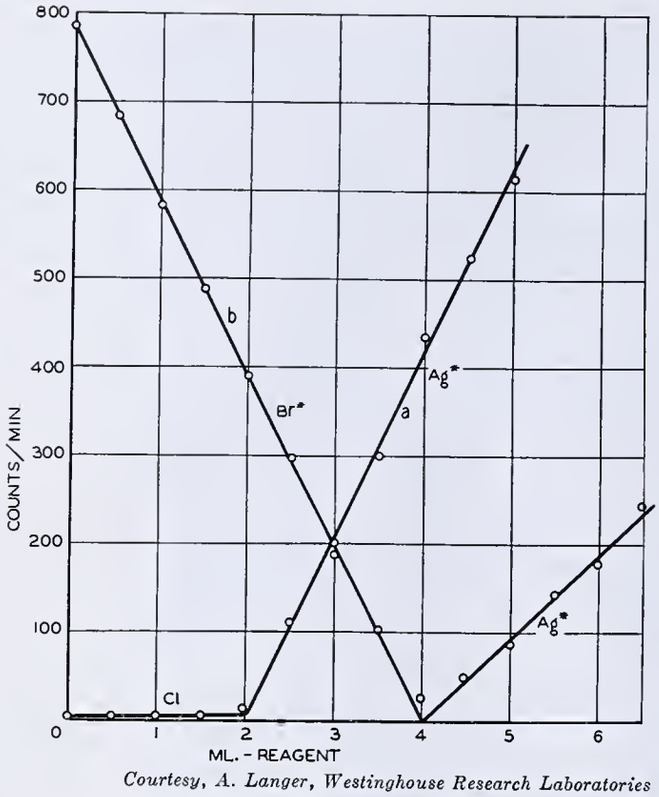
Many investigators view with suspicion instruments of such high sensitivity and suspect instability and temperamental behavior. It is encouraging to note that in connection with cosmic ray measurements a torsion type of fiber electroscope has been developed (*269*), with almost complete freedom from orientation effects, which could be mounted within 3 feet of the engine of a pursuit plane.

A typical installation is shown in Figure 180 in which the Lauritsen electroscope is mounted in an ionization chamber. An adjustable sample tray is mounted under the chamber, and a small lamp, operated from a filament transformer, is used to illuminate the fiber. A small power supply at the right supplies the direct current high voltage for charging the electroscope.

ELECTROMETER AND IONIZATION CHAMBER. A somewhat more sensitive arrangement of the integrating type is obtained with an ionization chamber to which an electrometer is connected.

The ionization chamber consists of an outer metal cylinder fitted with a well-insulated central, coaxial electrode. A high direct current potential (300 to 400 volts) is maintained across the pair. The potential is high enough to collect the ions before they can recombine (saturation current). Any gas may be used in the chamber; if very penetrating radiation is to be measured, the pressure of the gas may be increased. The ionization current is measured with a sensitive electrometer. Although any sensitive electrometer may be used, such as the Edelmann or Perucca, the present trend is in the direction of stabilized vacuum tube electrometers. In these arrangements, the ionization current is measured by a single-tube direct current amplifier.

Several papers have been published (*58*, *263*, *279*, *287*) dealing with the theory, construction, and operation of these circuits. Equations have been derived indicating the correct values for circuit constants in order to eliminate or minimize changes in the tube constants and fluctuations in supply voltages. In general, special "electrometer" tubes, such as the General Electric FP54, Western Electric D-96475, R. C. A. A-154, or Westinghouse DR+1-506 or 507, are used. These are specially constructed for the difficult problem of measuring very feeble currents from high-resistance sources (*196*). It is likely that the modern inverse-feedback amplifier, with its extraordinarily high stability, will soon replace the galvanometers which are at present used in tube electrometer circuits. However, the electrometer tube itself will still



Courtesy, A. Langer, Westinghouse Research Laboratories

FIGURE 187. RADIOMETRIC TITRATION CURVES

a. Chloride with radioactive silver nitrate
b. Bromide (with radioactive bromine) with radioactive silver nitrate

be used in the first stage, primarily for its transfer characteristics.

The combination of ionization chamber and electrometer is capable of measuring very weak samples. Tube electrometers can attain sensitivities of the order of 10,000 divisions per volt or charge sensitivities of the order of 5×10^{14} divisions per coulomb (*269*). This corresponds to radioactivities of about 10^{-4} microcurie (*247*).

Figure 181 illustrates a typical installation of an ionization chamber. The cylindrical brass container above the ionization chamber, with provision for evacuation and desiccation, contains the vacuum tube and part of the vacuum tube electrometer circuit. The remainder of the vacuum tube electrometer circuit is contained in the cabinet, above and behind which are located the galvanometer and scale.

GEIGER COUNTERS. The Geiger counter is sensitive to individual ionizing particles and depends for its high sensitivity upon ion-magnification. The ions produced by the primary particle are moving in an intense electric field and, by collision, produce thousands of other ions.

Three general classes of counters are recognized.

The Point Counter of Geiger (*88*, *236*) consists of a pointed wire placed axially within and insulated from a metal cylinder. A high potential of 1500 to 5000 volts is applied across the electrodes through a high resistance of the order of 10^9 ohms. The point is negative with respect to the cylinder. When an ionizing particle enters the counter a discharge takes place which continues until the potential drops below that necessary to maintain the discharge and the system returns to the normal sensitive state, ready for another count. The change in potential across the series resistor may be detected by a string electrometer or a pulse amplifier. The magnitude of the pulse is more or less independent of the number of ions originally produced by the ionizing particle.

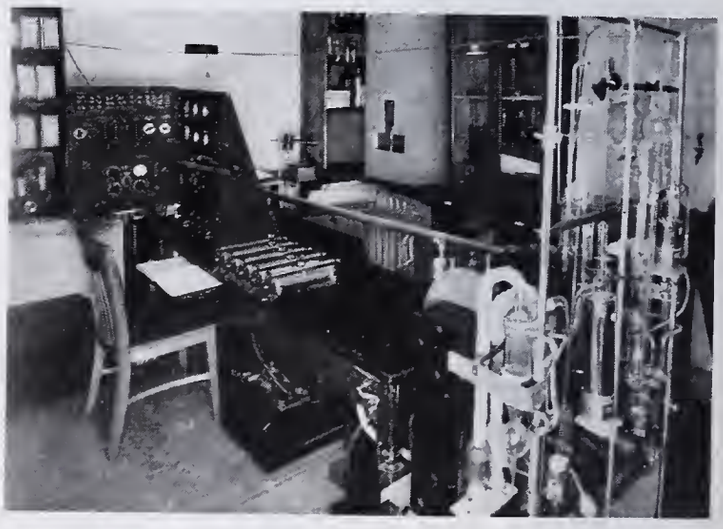
The Proportional Counter of Geiger and Klemperer (*89*) contains a small ball or sphere at the end of the wire, and in this case the wire is made positive with respect to the cylinder. With this arrangement the pulse is approximately proportional to the original ionization produced by the particle and one can distinguish between heavy particles, such as α particles, protons, and deuterons, and lighter particles such as electrons. A linear amplifier is used to measure the pulses.

The Zählrohr or Geiger-Müller Counter (*90*) provides a large area sensitive to ionizing particles. It consists of a cylinder and a coaxial wire between which a high potential is applied. The counter is filled with a suitable gas at reduced pressure. The bursts are detected with an alternating current pulse amplifier and can be made to operate mechanical counters.

Geiger counters have been the subject of many investigations and scores of applications. An excellent treatment from a very practical viewpoint is given by Neher (*204*, *269*). Because of their extensive use in cosmic ray research as well as in radioactivity, a large number of special circuits have been developed for accomplishing special tasks. A few of these may be mentioned.

1. For extremely fast counting, where the inertia of mechanical message-counters sets an upper limit, extensions of the Wynn-Williams (*307*) scale-of-two circuit may be employed. Here the actual count is divided exactly in half; two tubes count the bursts alternately. The adding of more scale-of-two circuits may be continued indefinitely, the final tube with its recorder reading 2^{-n} of the original pulse where n is the number of such circuits.
2. Special circuits have been developed to help the Geiger-Müller counter extinguish itself and thus increase the efficiency at high counting rates (*204*, *269*).
3. Coincidence-circuits have been developed (*228*) which record a count only if two or more G-M counters have discharged simultaneously. Since all counters register a minimal "background" count due to cosmic rays and radioactive contamination, an appropriate arrangement of coincidence-counters can be utilized to rule out or minimize these accidental registries.
4. Counting-rate meters have been developed (*72*, *94*) which will take randomly distributed pulses of any voltage, shape, and varying magnitude, convert them into uniform pulses (amplitude and duration) and feed them into an amplifier having a capacity-resistance tank circuit which performs a smoothing or averaging process. The output meter thus records the average rate of counting. Rates between 30 and several thousand counts per minute can be accommodated. The output can also be recorded with a recording galvanometer or milliammeter. An illustration of this type of instrument is shown in Figure 182. Preliminary details of its design and operation have been published (*151*) and a complete description will appear soon. A special bell-type beta-ray counter (*245*) with a pre-amplifier mounted in the cylindrical housing is shown resting on the top of the main amplifier cabinet. The pen recorder on the right provides a continuous ink record of the counting rate *vs.* time.

Another installation is shown in Figure 183. The lead shield on the right contains the Geiger-Müller counter tube. The unit



Courtesy, T. I. Taylor, University of Minnesota

FIGURE 188. MASS SPECTROGRAPH

in the center houses the vacuum tube high-voltage supply, amplifier, and scaling unit. In the left foreground is a Cenco impulse counter and behind it is a cathode ray oscillograph for monitoring purposes.

Applications. Atoms or the molecules containing them may be "tagged" with a small amount of their radioactive isotope and their subsequent behavior in any analytical scheme can be followed by measurements with one of the instruments described above. This presupposes the availability of a sufficiently long-lived isotope and a preparation of reasonably high initial intensity. A biological application of tagged atoms is illustrated in Figure 184, in which the counting-rate meter is used for a quantitative determination of the amount of radioactive iodine accumulated in the thyroid of a patient. In ordinary analytical separations, the method may be used to determine completeness of precipitation, coprecipitation, or distribution between solvents. Specific examples of these and many others are discussed in recent reviews (*76*, *121*, *247*).

Another method which has great possibilities for the detection of minute amounts of impurities is to subject the material to bombardment and produce the radioactive isotopes of the impurity. By means 6 parts per million of gallium in iron have been detected, small amounts of copper in nickel, and of iron in cobalt, etc. Again, one part of copper in 10,000 parts of silver has been detected by bombarding the silver with α particles (*150*).

An interesting combination of techniques has been described by Langer (*169*). Radioactive phosphorus P^{32} was converted to a soluble phosphate, several drops of which were added to a disodium phosphate solution. This was then used to perform several representative titrations (Ba^{++}, Pb^{++}, Th^{++++}, Mg^{++}, UO_2^{++}). After each addition of the reagent, the filtered solution was sucked up into a special chamber surrounding a Geiger-Müller counter. A counting-rate meter (*94*) was used to measure the activity. The end points could be determined accurately, by the intersection of the activity curves.

Figure 185 shows the apparatus used for these measurements, and Figure 186 some examples of liquid counters suitable for analytical work. Through the courtesy of Dr. Langer, the unpublished results of some further work are shown in Figure 187.

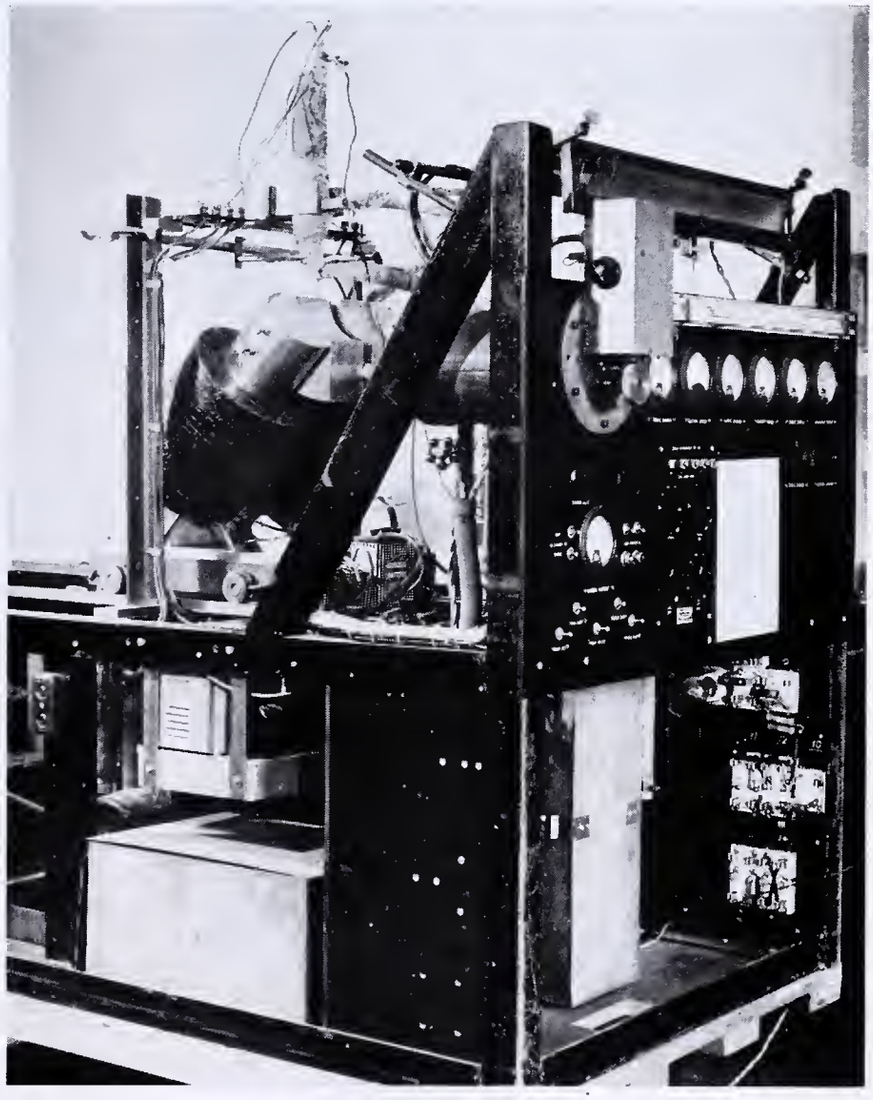
Conclusion. It is difficult to appraise the significance of this mode of analysis; although the field of artificial radioactivity is but seven years old, nearly six hundred papers have been published. The main interest lies in the nuclear studies, and the analytical applications have been relatively few but nevertheless very important and revolutionary. For some time to come, the radioactive isotopes as "reagents" will continue to be scarce, for their preparation is very costly. On the other hand, there are few fields of investigation so well provided with refined and sensitive instruments. Nuclear physics and chemistry have attracted the most brilliant and gifted investigators and ample support for their work has been freely given. Such is the tempo that any textbook on the subject is likely to be out of date before it leaves the press.

Mass Spectrographic

The mass spectrometer or spectrograph is a device in which gases or vapors can be subjected to bombardment by electrons of controlled velocity. The molecular fragments which are ionized can be sorted out by combined electric and magnetic fields and brought to a focus on a photographic plate or on an exit slit. In the case of the spectrograph the ion beam is brought to a focus in the plane of the photographic plate and the resulting mass spectrogram contains lines, the spacing of which depends upon the e/m values of the respective ionic species.

In the mass spectrometer the electric or magnetic focusing fields are so adjusted as to bring one species after another to a fine exit slit, behind which there is placed a Faraday cage which collects the ions. This positive ion current is a measure of the abundance of the particular ionic species that has reached it. By either of the methods it is possible to sort out the molecular ion fragments according to their relative masses and abundance.

This very valuable technique is based on almost half a century of fundamental work by J. J. Thompson, F. W. Aston, Dempster, Smyth, Hogness and Lunn, Bleakney, and others. Among the many contributions to physical science which this technique has made may be mentioned the determination of the relative abundance of naturally occurring isotopes, from which "mass defects" can be computed.



Courtesy, Westinghouse Research Laboratories

Figure 189. Portable Mass Spectrometer

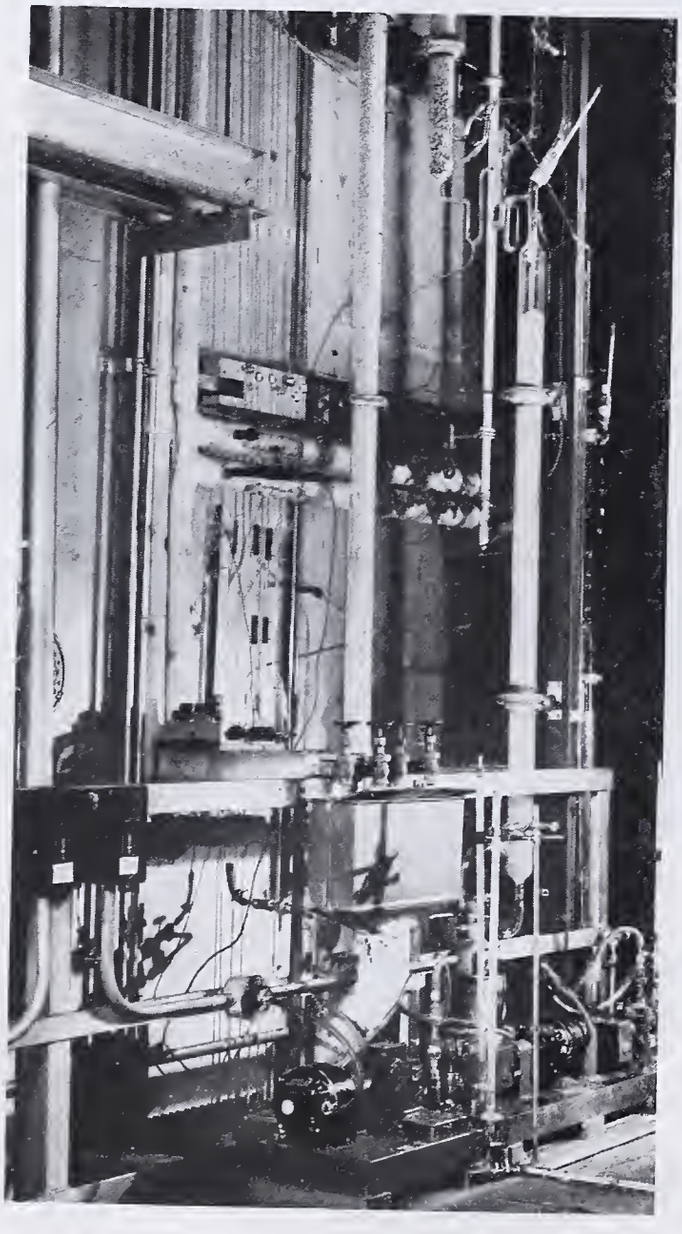
From the analytical view point its value resides in the following possibilities:

1. In the use of stable isotopes for tracer work, it is the most accurate and reliable means of analysis.
2. It is able to determine small traces of certain gases in mixtures with a high degree of certainty and identification.
3. It is adaptable to very small amounts of material, since the total gas pressure maintained in the spectrometer is of the order of 10^{-4} mm. of mercury.
4. Continuous changes in composition can be followed.

In general, the sample must be in the form of a gas or a vapor and although it is possible to work with any of the metals in the vapor form, this, as might be imagined, is beset with certain technical and manipulative difficulties. In some cases, such as the alkali metals, salts can be heated, resulting in the thermionic emission of the metals as positive ions, which can then be resolved by the electric and magnetic fields of the spectrometer.

Instruments. Two handsome examples of the mass spectrometer may be discussed briefly.

In Figure 188 is shown the mass spectrometer used by Taylor at the University of Minnesota. It is a Dempster 180° focusing type, designed to measure the abundance ratio of isotopes. It is similar in construction to those described by Bainbridge (*10*) and Brewer (*28*). The analyzing chamber between the poles of the magnet is equipped with an interchangeable ground-glass joint to facilitate the use of different positive ion sources. A small platinum disk impregnated with an alkali element and heated with a tungsten filament serves as a source of positive ions for these elements. Gaseous substances can be admitted to the system through a fine capillary tube, after which they are ionized by bombardment with a beam of electrons. The ions are accelerated into the magnetic field by use of a high-voltage rectifier unit. The resolved positive ion current is measured with a F. P. 54 tube electrometer circuit of the DuBridge and Brown type (*58*).



Courtesy, Eastman Kodak Co.

Figure 190. Apparatus for Separation of Isotopes by Chemical Exchange

The spectrometer unit with its associated magnet and high-vacuum pumps appears on the right. The electrometer unit and control panels are at the extreme left, sufficiently removed from the local field of the magnet and connected with the Faraday cage of the spectrometer by the long horizontal tube which is carefully shielded and grounded.

The portable mass spectrometer shown in Figure 189, which was designed and built at the Westinghouse Research Laboratories by John A. Hipple, Jr., embodies all the latest improvements in this field. A tentative description (August 1, 1941) has been prepared by Westinghouse. The deflecting magnet is clearly shown in the upper left-hand portion, with the inlet tube and gas ionizing mechanism rising vertically above the pole pieces. The magnet is the 90° type, affording a shorter ion path, and since this reduces the number of collisions of the ions with the gas in the analyzer, somewhat higher gas pressures than usual can be tolerated. The use of a vertical ion source affords an experimental simplification in the ease with which new tubes can be sealed on and filaments can be replaced where necessary. Theoretical computations based on the instrument parameters indicate a resolution of 109, according to which the instrument should resolve mass 108 from mass 109.

The predictions are verified in representative analyses, which show as expected that the resolution is of this order. Typical curves taken on carbon dioxide show the three isotopes of oxygen, O^{16}, O^{17}, and O^{18}, and the two isotopes of carbon, C^{12} and C^{13}, which values are evident in curves showing a mass of 44, 45, and 46. Another test shows the mercury isotopes, Hg^{204} and Hg^{202}, which represent two mass units in 200 in the resolution expected from the geometry of the apparatus. The remaining mercury isotopes, which are only one mass unit apart, are not completely resolved. Very useful information has already been obtained with this instrument. Information is available in the literature for the following molecules: methane, ethane, acetylene, ethylene, propane, propylene, allylene, butane and isobutane, benzene, sulfur dioxide, carbon tetrachloride, chloroform, ammonia, carbon bisulfide, cyanogen, methyl alcohol, ethyl alcohol, nitrous oxide, bromine, hydrocyanic acid, carbon dioxide, carbon monoxide, water, hydrogen, oxygen, and nitrogen peroxide.

The sensitivity for analysis is expressed as the smallest amount of the least abundant component mass that can be detected in the presence of the most abundant component. One example may be mentioned. The instrument has been used to measure as little as 0.001 per cent of oxygen in nitrogen. The entire equipment is placed on a truck and the only outside connection required when the apparatus is in place is 110-volt alternating current and water connections for cooling the pumps. Further details are available in the Westinghouse publication, and it is a privilege to be able to present these meager details of an instrument, from which much important and useful information will undoubtedly be forthcoming in the near future.

Applications. The vast program which has been in progress for some time on the separation and utilization of naturally occurring isotopes has been recorded in many places. Starting with the discovery of deuterium by Urey in 1932, there has been rapid progress in the separation or enrichment of other useful isotopes, such as Urey's methods for C^{13} from the more abundant C^{12} and also of N^{15} in quantities sufficient to make these substances available for biological tracer work (see also *223*). His methods (*137*) for enrichment based on the exchange principle have proved most useful (*268*, *273*). A typical example of columns for these studies is shown in Figures 190 and 191. Some examples of the use of these isotopes as tracers have been given by Rittenberg (*35*, *77*, *244*), and many others are currently recorded in the literature.

Courtesy, Eastman Kodak Co.

FIGURE 191. APPARATUS FOR SEPARATION OF ISOTOPES BY CHEMICAL EXCHANGE

Earlier investigations in the isotope field employed a great variety of methods, mostly refinements of density measurements, but at the present time the mass spectrograph despite its complication is regarded as the most reliable and accurate tool for the purpose.

Acknowledgment

The author is greatly indebted to Etzio Giolito, the Misses Evelyn Gamble and Marianne LaGana, and the editorial staff of INDUSTRIAL AND ENGINEERING CHEMISTRY for expert and indispensable aid in the preparation of the manuscript.

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Chromate Corrosion Inhibitors in Bimetallic Systems

Compared by a Scoring Method Based on Visual Observations

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Increasing amounts of water being used for air conditioning, washing, cooling, and condensing raise a problem in many localities in respect to water supply and sewage disposal. Present practice is tending toward recirculating systems to save the water and the use of corrosion inhibitors to save the systems. It is well known that the corrosion of many metals is greatly reduced by the presence of sodium chromate or sodium bichromate (*3*, *4*), but there are almost no data in the literature showing the relative efficiency of these chemicals for the retardation of bimetallic corrosion in ordinary recirculating water systems.

Comparisons are presented in this report for iron, galvanized iron, tinned iron, copper, brass, and aluminum, both alone and in contact with each other. The corrosion of these metals in various water systems was greatly retarded or completely inhibited by sodium chromate at the concentrations indicated. For some combinations sodium bichromate was equally satisfactory, but in general it was not so efficient as the chromate. In addition to prolonging the life of equipment, the formation of slimes may be avoided.

MANY corrosion processes may be followed by weight changes, provided precautions are taken to ensure that corrosion products either remain entirely on the specimens or are completely removed, without removal of uncorroded metal. Such data, however, may require interpretation by corrosion specialists.

For instance, iron may increase in weight because of formation of rust scales. Aluminum increases in weight, but the oxide film may be protective. In the presence of an oxidizing passifier such as sodium chromate, Hoar and Evans (*1*, *5*) have noted the formation of a dense inert protective film containing chromic oxide, Cr_2O_3. Others have noted the formation of protective films, such as zinc chromate on galvanized surfaces. Wilson and Groesbeck (*6*) have used salt-spray methods for testing corrosion inhibitors in air-conditioning equipment, and report that chromates and bichromates are the most efficient.

Corrosion tests conducted at this laboratory have revealed little relationship between weight changes in bimetallic systems and the practical value of chromate inhibitors. Table I gives some weight-change data illustrating how difficult they are to evaluate even for very simple systems in the presence of chromates.

Without visual inspection it is not apparent from Table I that the chromate completely inhibited corrosion. With bimetallic systems weight changes have been found to be even more complex and almost impossible to interpret without visual data. On the other hand it has been noted that the impression formed on visual examination corresponds in most instances to practical behavior. This does not apply to corrosion which produces concealed defects such as the intercrystalline corrosion of aluminum, the graphitizing of cast iron, or the caustic embrittlement of steel. Concealed corrosion can be evaluated best by physical measurements, such as loss of tensile strength, or by microscopic examination. Depth of penetration methods are also of practical value.

Various electrochemical methods that have been proposed provide fundamental assistance in studying the mechanism of corrosion, but require great skill and theoretical knowledge of the factors influencing corrosion. They are difficult because of innumerable local potential differences between different areas of the same metal, and between corrosion products. With dissimilar metals in contact in aqueous solutions—which is common from the practical viewpoint—the situation is particularly complex, resulting in electrochemical processes which may either intensify or retard corrosion.

Visual Scoring

This method is based on simple observations and the weighted scoring of characteristic corrosion criteria, which may be expressed numerically, and can be duplicated by different observers with a degree of precision satisfactory for practical purposes. The method may be varied to simulate

TABLE I. WEIGHT CHANGE AFTER 6 MONTHS AT 70° F.

	Aluminum	Iron	Galvanized Iron	Tinned Iron
Tap water	+0.123	−0.849	−0.564	−0.161
500 p. p. m. Na_2CrO_4	+0.021	+0.012	+0.017	+0.008
500 p. p. m. $Na_2Cr_2O_7$	+0.004	+0.007	−0.015	−0.020

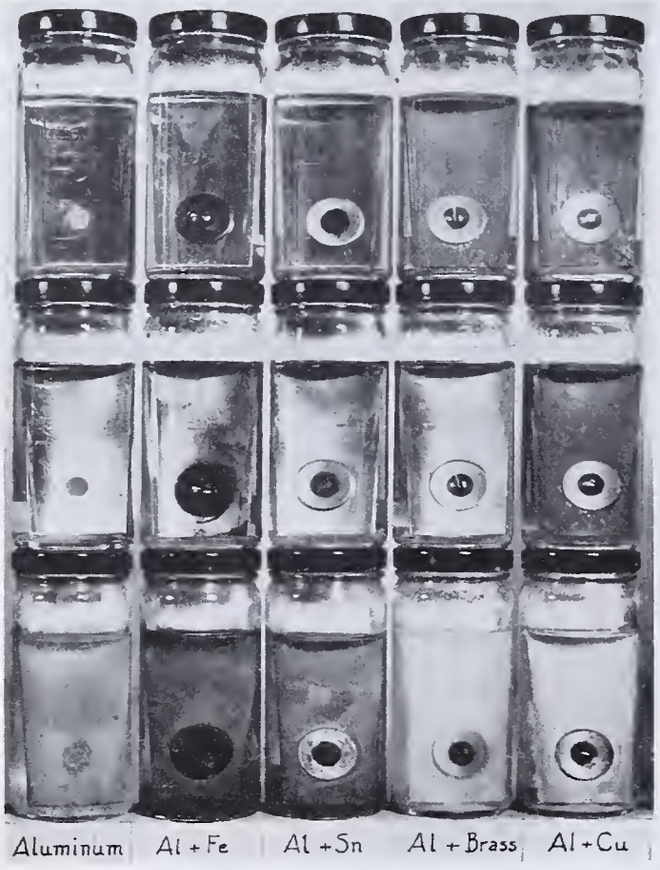

FIGURE 1. CORROSION TESTS

various practical conditions. Scoring may be reweighted to obtain comparisons for different applications.

Illustrative of a way in which corrosion criteria may be weighted for an air-conditioning system, cloudiness may be allowed 4 points out of a possible 100. Low scoring for cloudiness is indicated because it may not be harmful. If a precipitate settles, it may result in some harm and should be given a greater weight. If the precipitate is such that it may clog the system, a still greater weight is indicated. If the general appearance score is zero, the precipitate and cloudiness scores also will be close to zero. Other corrosion criteria may be selected and weighted in a similar manner.

The accompanying data further illustrate the scoring method. Forty points were allowed for the condition of each of the metals in contact; 20 points for the corrosion products in the liquid. The corrosion of each metal was scored on the basis of five types of observation: discoloration, roughening, pitting, depth of pits, and general corrosion. By general corrosion are meant the nature and distribution of corrosion, the general impression of the observer, and unclassified factors. Evidence of corrosion in the liquid was scored in a similar manner based on cloudiness, precipitation, and general appearance. Under general appearance of the liquid are included unclassified variables, the nature of the precipitate, and its probable harmful effect in a recirculating system.

The weightings employed in this report, shown in Table II, are arbitrary but logical and illustrate a practical application. Different weightings, however, are used for different applications. For instance, different weightings are indicated for an automobile cooling system than for an air washer. If discoloration or slimes are unimportant they may be disregarded. For some purposes the entire liquid score is disregarded. The scoring procedure in this laboratory is for the observers to check all of the descriptive terms on a report form. These data are assembled and weightings assigned according to the particular purpose of the corrosion project. The nature of the scoring method is such, however, that ordinary changes in weightings seldom result in important changes in the relative corrosion order. Of course the same weightings must be used in comparing corrosion scores.

A general idea of the protection efficiency may be obtained from the sum of the liquid and metal corrosion scores, but for many purposes it is best to consider them separately. In a bimetallic system, 100 represents perfect inhibition; with single metals, 60 indicates complete protection. A limitation of this method is that it does not afford a numerical comparison between monometallic systems and bimetallic systems, except under special conditions. There are technical difficulties in making comparisons between these systems due to differences in the nature of the initial corrosion centers or pits. The corrosion trend in monometallic systems is slower in developing. This was particularly evident with aluminum and to a lesser extent with zinc. For aluminum in tap water, the corrosion scores were better after 18 months than after 6 months. These changes appear to be due to the initial formation of many corrosion centers which are heavily weighted, because in most systems even small pits are an indication of the start of severe corrosion. It happens, however, that these initial pits, instead of deepening, either spread until they join to form a fairly uniform and resistive surface or change in light reflection so as to become invisible except under special illumination and low magnification. The procedure employed in this report is limited to corrosion criteria which can be seen by unaided visual inspection.

TABLE II. WEIGHTINGS

For Condition of Metal	
Discoloration	None (3), slight (2), moderate (1), considerable (0)
Roughening	None (4), slight (3), moderate (2), considerable (0)
Pitting	None (9), slight (6), moderate (3), considerable (0)
Depth of pits	None (12), shallow (9), moderate (6), deep (3), very deep (0)
General corrosion	None (12), slight (9), moderate (6), considerable (3), very bad (0)
For Condition of Liquid	
Cloudiness	None (4), slight (3), moderate (2), considerable (0)
Precipitate	None (8), slight (5), moderate (2), considerable (0)
General appearance	Good (8), fair (6), poor (4), bad (2), very bad (0)

It has also been found, when inhibitors are absent, that oxygen is so rapidly consumed as to cause corrosion to depend on the rate at which additional oxygen is obtained from the air, thus making numerical comparisons between some uninhibited systems meaningless—beyond the general observation that it is very severe as compared to inhibited systems. Irrespective of the explanation, a study of about a thousand corrosion record sheets indicates that technical difficulties in making numerical comparisons are very much less when inhibitors are present. The corrosion scores of all inhibited systems are given in Table III. Scores are also given for uninhibited bimetallic systems for purposes of general comparison, although they may be only approximately correct because of variations in oxygen deficiency. Corrosion scores have not been assigned to monometallic systems in tap water, but it can be seen from Figures 1, 2, and 3 that corrosion was severe in comparison with the inhibited systems. If numerical comparisons are desired between these systems a different procedure is indicated, involving aeration; but this is beyond the purpose of the present investigation which is to evaluate the protective efficiency of chromate inhibitors.

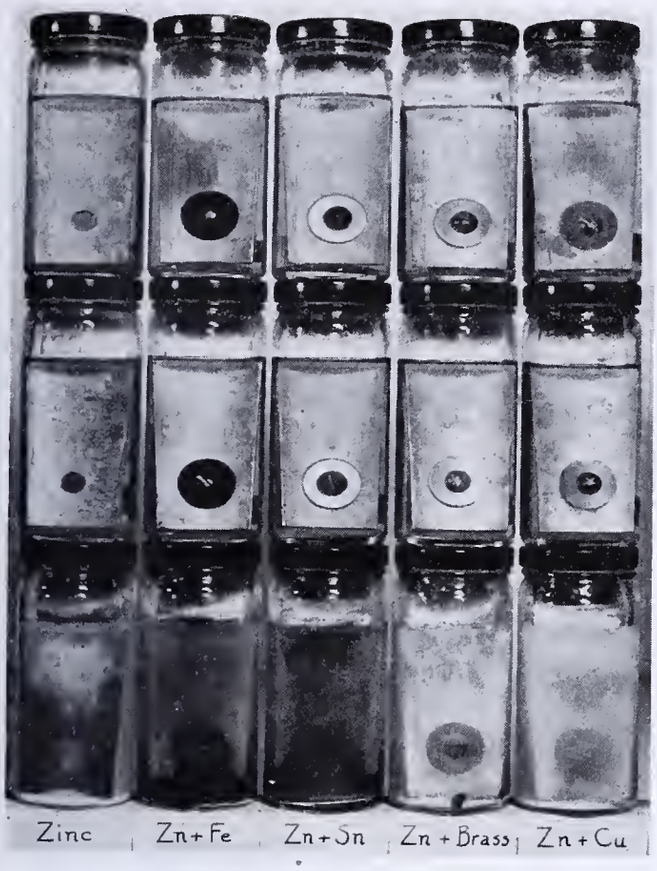

FIGURE 2. CORROSION TESTS

Experimental Data

Testing conditions which are not specified were identical for all specimens. "Tap water" refers to Baltimore city water. "Chromate" and "bichromate" refer to solutions of sodium chromate, Na_2CrO_4, and sodium bichromate, $Na_2Cr_2O_7.2H_2O$, at concentrations equivalent to 1000 parts of Na_2CrO_4 per million. All regular tests were run in closed 8-ounce screw-cap glass jars, without agitation or aeration. Some aerated tests also were run.

The aerated tests indicated that corrosion in the presence of sodium chromate is essentially the same in closed as in aerated systems. If no chromate is present the corrosion is very much more rapid when aerated. Since there are varying degrees of aeration in practical applications, and since aeration is often the rate-making factor when chromates are absent, the most conservative way of evaluating chromate corrosion inhibitors is without aeration. In other words, the conditions of test used in this investigation represent the minimum relative retardation of corrosion afforded by the chromate. There are many conditions of aeration where the merit of the chromate is considerably greater than that indicated. There was some access of air when the test samples were inspected, but all were handled in an identical manner, and the admission of air was less than under any ordinary condition of practical use. The black appearance of the corrosion products on the iron test panels in tap water (Figure 3) indicates insufficient oxygen.

The photographs (*2*), Figures 1, 2 and 3, show the general appearance of the corrosion tests after 6 months at room temperature.

The top rows contain sodium bichromate, the middle rows sodium chromate, and the bottom rows tap water. The bolts and washers show the effect of contact with a dissimilar metal. Markings on the photograph show the plate metal first, followed by the washer metal. In each test the bolts and nuts are the same metal as the washers. Zinc refers to heavily galvanized iron plates. Tin refers to heavily tinned iron plates. Both galvanizing and tinning were done by double-dipping after cutting and drilling. Brass refers to a commercial grade, analyzing about 60 per cent copper, 40 per cent zinc. Copper and aluminum refer to the commercially pure metals, analyzing about 99.9 per cent copper and 99.0 per cent aluminum. The iron panels were cut from a mild steel sheet known as type A tank (about 0.1 per cent carbon). All panels were uniformly polished, cleaned, and inspected for defects prior to testing.

The panels tested at 160° F. were placed in thermostatically controlled ovens with uniform heat distribution. No light was admitted to the ovens. The room-temperature test panels were shielded from direct sunlight and were inspected at frequent intervals up to 18 months.

The principal data on which this report is based are the composite observations of four men for the same panels shown in the photographs. Several hundred similar tests were run. There were slight variations between tests started at different times, but on the whole there were no discrepancies between other corrosion series and those reported here. There were some variations in details of scoring by different observers, but the total corrosion score of each observer for any particular condition was essentially the same, as was the composite score. The corrosion scores of the monometallic systems did not prove to be a measure of the relative merit of the inhibitors because either sodium chromate or sodium bichromate gave such perfect inhibition that comparison of their scores was almost meaningless.

The tabulated data for iron and copper in contact illustrate the scoring method. The data for the aluminum series, zinc series, and iron series present the corrosion scores for the more important metal combinations. The plates and washers may be considered separately or in combination, depending on the purpose of comparison.

HOT WATER TESTS. Corrosion inhibition in hot water roughly paralleled that at room temperature. As would be expected, corrosion is more rapid. At the end of 6 weeks at 160° F. the corrosion scores of most of the bimetallic test panels dropped to figures comparable to 6 months at 70° F. With combinations

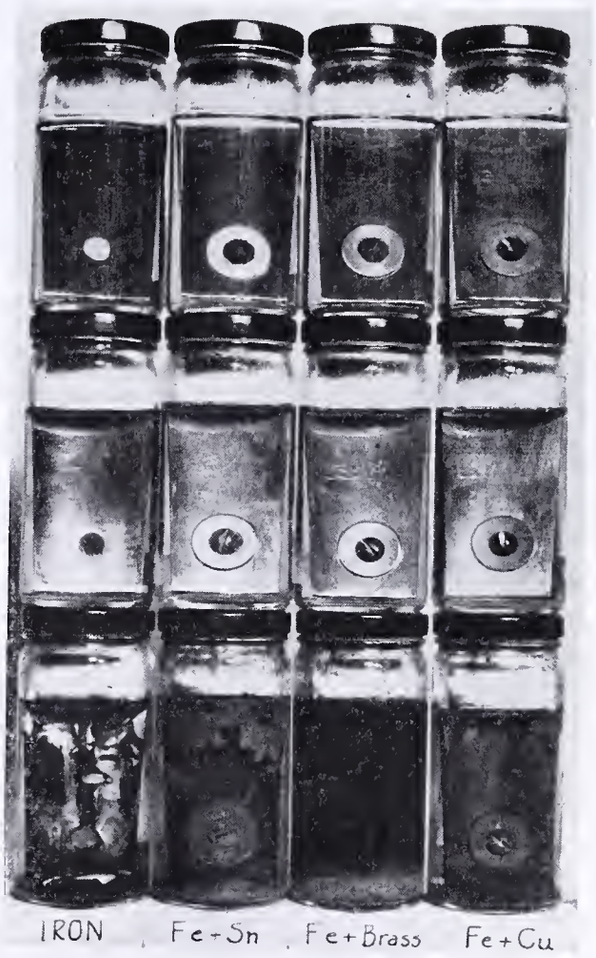

FIGURE 3. CORROSION TESTS

TABLE III. SCORING METHOD

	Tap Water	Chromate	Bi-chromate
Iron and Copper in Contact 6 Months at 70° F.			
Iron Plates			
Discoloration	Considerable	None	Slight
Roughening	Considerable	None	None
Pitting	Moderate	None	None
Depth of pits	Deep	None	None
General corrosion	Very bad	Slight	Slight
Copper Washers			
Discoloration	Slight	Slight	Slight
Roughening	None	None	None
Pitting	None	None	None
Depth of pits	None	None	None
General corrosion	Slight	None	None
Liquid			
Cloudiness	Considerable	None	Slight
Precipitate	Considerable	None	Slight
General appearance	Bad	Good	Fair
Plate score (40 = perfect)	6	37	36
Washer score (40 = perfect)	36	39	39
Liquid score (20 = perfect)	2	20	14
Protection efficiency (100 = perfect)	44	96	89
Aluminum Systems[a], 6 Months at 70° F.			
Aluminum Plates Alone			
Plate score	X	40	40
Liquid score	X	20	20
In Contact with Iron Washers			
Plate score	29	39	37
Washer score	20	38	25
Liquid score	5	20	14
In Contact with Copper Washers			
Plate score	10	19	14
Washer score	34	39	40
Liquid score	9	11	7
In Contact with Brass Washers			
Plate score	13	30	17
Washer score	34	39	33[b]
Liquid score	9	17	9
In Contact with Tin Washers			
Plate score	23	40	40
Washer score	15	40	39
Liquid score	9	20	20
Zinc Systems[a], 6 Months at 70° F.			
Galvanized Iron Plates Alone			
Plate score	X	40	39
Liquid score	X	20	20
In Contact with Plain Iron Washers			
Plate score	20	39	35
Washer score	34	37	37
Liquid score	4	17	14
In Contact with Copper Washers			
Plate score	9	36	28
Washer score	35	39	22[c]
Liquid score	4	14	14
In Contact with Brass Washers			
Plate score	20	40	29
Washer score	39	40	26[c]
Liquid score	8	20	16
In Contact with Tinned Washers			
Plate score	11	40	39
Washer score	14	39	39
Liquid score	7	20	17
Iron Systems[a], 6 Months at 70° F.			
Iron Plates Alone			
Plate score	X	40	37
Liquid score	X	20	20
In Contact with Copper Washers			
Plate score	6	37	36
Washer score	36	39	39
Liquid score	2	20	14
In Contact with Brass Washers			
Plate score	18	37	36
Washer score	39	39	36[b]
Liquid score	2	20	17
In Contact with Tinned Washers			
Plate score	21	37	37
Washer score	31	39	35
Liquid score	2	17	17

[a] X denotes that corrosion was severe in comparison with inhibited systems, as can be seen in photographs, but method employed does not give directly comparable figures for monometallic systems in tap water.

[b] Slightly lower score of brass washers in presence of sodium bichromate is due chiefly to discoloration, which may be disregarded for many purposes.

[c] Low score of copper washer in contact with zinc in presence of sodium bichromate is due to formation of a hard, rough, tightly-adhering layer of crystalline zinc compound over surface of copper, causing it to be reported as considerably roughened and pitted. Scraping off this deposit after 18 months revealed that copper had not actually been attacked. The scoring method is undoubtedly too severe in this instance, but shows that an undesirable condition exists when sodium bichromate is used in this system rather than sodium chromate. Somewhat similar conditions exist with brass washers.

of aluminum-copper and iron-copper, sodium chromate was more beneficial in hot water than in cold, whereas with aluminum-iron, zinc-iron, and zinc-copper combinations, sodium chromate was a little more effective at room temperature.

CHROMATE CONCENTRATION TESTS. Owing to accelerated corrosion at 160° F., a series of tests was run at this temperature, with iron plates in contact with copper washers, using triple proportions: 3000 parts per million. There was no corrosion after 8 weeks at 160° F. in the presence of sodium chromate, and only slight corrosion in the presence of sodium bichromate. Corrosion also was negligible with sodium chromate at a concentration of 1000 p. p. m. In other words, the protection afforded by sodium chromate was about the same at both concentrations. Lower concentrations were not included in this series, but there are ample records at this laboratory indicating that under most conditions concentrations of 200 to 500 p. p. m. provide good protection. Concentrations below 100 p. p. m. protect aluminum and zinc, but may cause stimulation of corrosion with iron systems.

REGULATED pH TESTS. A set of corrosion tests was run with iron plates in contact with copper washers, with the alkalinity of the sodium chromate adjusted to pH 7.0 by addition of sodium bichromate, the total parts per million of chromium being kept the same. Tests were for 8 weeks at 160° F. using a concentration equivalent to 1000 p. p. m. Another set was run with 3000 p. p. m. For further comparison, a third set was run at the same time without adjusting the alkalinity. These tests indicated that adjustment to pH 7.0 does not result in an impressive difference. If anything, sodium chromate without adjusting alkalinity gave slightly better protection.

AERATED TESTS. A set of aerated corrosion tests was run with the iron systems at 70° F. The air was drawn from outside the building by means of a water aspirator. It was well filtered, water-washed, and supplied at a low steady pressure. The air was bubbled through all test samples continuously at a slow uniform rate. All samples were tested at the same time and aerated in an identical manner. For comparison a closed set also was run at the same time without aeration but with other conditions identical. The corrosion of all the aerated test panels in tap water was very much faster and more severe. The behavior of the aerated samples in the presence of sodium chromate was the same as the corresponding nonaerated test panels.

EIGHTEEN-MONTH TESTS. The bimetallic systems retained essentially the same corrosion order, which was evident a few days after starting the tests, up to the time the photographs were taken 6 months later. Rescoring after 18 months showed essentially the same order as at 6 months. There were no signs that the sodium chromate had lost its effectiveness. In some cases the protection was better after 18 months than after 6 months.

PRACTICAL TRIALS. In trials with several small York air-conditioning units (34 × 37 inch washers), one Carrier (26 × 30 inch washer), and one large Sturtevant air-washer (60 × 60 × 84 inch spray chamber), weekly observations over a period of 18 months indicated that the use of sodium chromate not only prevented corrosion but also eliminated the formation of organic slimes. The nature of the microorganisms destroyed was not

investigated. Two new units without chromate accumulated a large amount of slime during the same period. One old unit which had accumulated slime and rust prior to the test completely cleared during the 18-month chromate treatment. Because of fluctuations in overflow the concentration of sodium chromate varied between 100 and 500 p. p. m. Each week it was adjusted to 500 p. p. m., on the basis of yellow color which was found satisfactory for practical purposes. Although chromate replacement is frequently due to mechanical loss through the water overflow, some consumption is due to the oxidation of organic material removed from the air. Many bacteria, molds, and accompanying odors are destroyed. Sodium bichromate is a little more reactive than sodium chromate, and consequently has a shorter life if much organic material is present. The foregoing results have been substantiated by other practical observations extending over 4 years, including several large air-conditioning units.

Summary

Visual scoring is adapted to the evaluation of chromate corrosion inhibitors. Results may be duplicated with an accuracy sufficient to be of practical value.

Sodium chromate is effective in retarding, or completely inhibiting, the corrosion of many bimetallic systems in water. Sodium chromate and bichromate are effective in retarding corrosion in most monometallic systems. Sodium bichromate also reduces corrosion in many bimetallic systems, but it is not so generally effective as sodium chromate.

Aluminum and copper, aluminum and brass, and zinc and copper combinations are difficult to inhibit. With these combinations, sodium chromate is very helpful but not completely inhibitive.

The relative retardation of corrosion by sodium chromate is essentially the same in hot water as in cold.

Neutralizing the alkalinity of sodium chromate to pH 7.0 does not result in any impressive differences. If anything, sodium chromate is more effective at its natural alkalinity of about pH 8.5.

Although the rate of corrosion of most systems is greatly accelerated by aeration, there is no important difference between aerated and closed systems when sodium chromate is present.

Increasing the concentration of sodium chromate slightly improves the protection, but this is not indicated except as a factor of safety when recirculating water is subject to dilution. No harm results from excess sodium chromate. Amounts less than about 100 p. p. m. should not be used for iron systems.

Sodium chromate retains its inhibitive properties until lost from the system, either mechanically or by chemical reduction. For many practical purposes its depletion may be judged by loss of color.

Acknowledgment

The writer wishes to take this opportunity to acknowledge the assistance and helpful suggestions of the Baltimore staff of the Mutual Chemical Company of America, particularly O. F. Tarr, W. H. Hartford, and D. F. Altimier, and to express appreciation to G. H. Young, E. Ward Tillotson, W. A. Hamor, and others associated with Mellon Institute for many thoughts and constructive criticisms.

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Controlling Apparatus to Eliminate Waste of Water in Using the Ordinary Filter Pump

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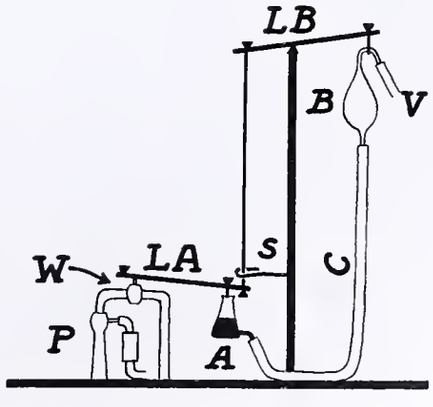
THE cost of operating the ordinary aspirator or suction pump is usually concealed in a single water bill which includes more legitimate uses as solvent, diluent, etc. The cost for a single aspirator may be on the order of thirty dollars per year, depending upon several obvious factors. For filtration, the removal of a supernatant fluid from a precipitate, the removal of used samples and reagents from such apparatus as the Van Slyke blood gas pipet, and many distillations under reduced pressure where rather large variations in pressure are not detrimental, a closed suction system with automatic control of the supply of water to the aspirator has proved satisfactory. The controlling apparatus shown in the accompanying figure has been found suitable for the purpose. It is highly reliable, having been in operation night and day for months during the past 3 years without attention or interruption.

P indicates the filter pump, connected to the vacuum line through a check valve (Bunsen valve), *W* is a whistle valve, and *V* is a rubber tube connecting flask *B* with the vacuum line. In operation, mercury in flask *A* depresses lever *LA*, opening the whistle valve and starting the aspirator. Increasing vacuum in the system "lifts" mercury from flask *A* to flask *B* until the shift



of weight is sufficient to depress *LB* and lift *LA*, shutting off the water. The two flasks and the rubber tubing connecting them, *C*, form a manometer in which the difference in mercury levels is a measure of the vacuum. The reduction in pressure in the system at the time of cutoff can be adjusted by raising or lowering the flasks in relation to the levers and to each other.

A controlling device for a suction system must have some lag in operation to avoid excessively frequent opening and closing and a tendency to chatter around the point of equilibrium. The lag in this apparatus is rather large, about 100 mm., but for many purposes this is not objectionable. If at any time the reduction below atmospheric pressure is not great enough, an inlet in the system may be opened to start operation of the aspirator. If uniform maximum suction is wanted, a pinchclamp may be placed on the connecting tube when flask *A* is filled with mercury, to prevent cutoff. When the suction system is not in use, the controlling apparatus may be left in the off position by placing the clamp on the connecting tube when flask *B* is full.

As shown, the entire control apparatus is accessible and in view for occasional inspection. It would be possible to use a similar device which had only one lever, the two flasks being hung from opposite ends. However, either one flask and the connecting tube would extend below the table or the piping and whistle valve would have to be elevated to several feet above it.

The material required is easily available, and construction is easy. The levers are of 5 × 16 mm. (0.187 × 0.625 inch) stock. The connecting rod between the levers is of 6-mm. (0.25-inch) cold-rolled steel. The support for the upper lever, *LB*, is 13-mm. (0.5-inch) pipe in a standard floor flange screwed to the table. All pivots (shown conventionally by black triangles) are 6-mm. (0.25-inch) bolt or 6-mm. rod, turning in drilled holes, except those above the flasks which are of iron wire hanging in V-shaped notches. The stop, *S*, is made of a piece of 6-mm. cold-rolled steel rod threaded and screwed into the support part of an ordinary ring-stand clamp. For connection with the heavy-walled rubber tubes, *C* and *V*, large capillary tubing (2 mm. in inside diameter) is fused to each of the 300-ml. flasks as shown. The capillary tubing is strong and tends to prevent violent surge in the mercury. The rubber connecting tube, *C*, must extend low enough to prevent mercury from being drawn below its lowest point and admitting air. A 13-mm. (0.5-inch) whistle valve is used. The upper lever, *LB*, extends 127 mm. (5 inches) to the left and 229 mm. (9 inches) to the right. The lower, *LA*, is 305 mm. (12 inches) long with 19 mm. (0.75 inch) between the pivot and the stem of the whistle valve. The support is 92 cm. (36 inches) high. It is desirable to place a trap in the line below *V* to prevent dirty liquid from getting into *B*, and the trap is essential to catch mercury, if the suction system is of copper tubing. A rubber stopper between *LA* and *S* cushions the blow when the lever hits the stop.

The Molybdenum Blue Reaction

A Spectrophotometric Study

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UNDER suitable conditions molybdates react with phosphates, arsenates, silicates, and certain other salts or acids to form heteropoly compounds, such as ammonium molybdiphosphate, $(NH_4)_3[P(Mo_3O_{10})_4]$, or molybdisilicic acid, $H_4[Si(Mo_3O_{10})_4]$. These complexes, after controlled reduction to give molybdenum blue, serve for the colorimetric determination of various reducing agents or of elements which function as central atom in the complex anion, such as phosphorus, arsenic, or silicon. The procedure must be carefully controlled, as the excess molybdate reagent itself may be reduced to molybdenum blue.

In view of the analytical uses of such reactions, it seemed of interest to make a spectrophotometric study of the processes in order to learn more about their nature, the effect of different variables, and the merits of the several procedures.

Previous Work

THE REDUCTION PROCESS. The nature and the composition of the material obtained on reducing simple molybdates are uncertain (*32*). Low concentrations of reactants yield what appears to be a solution, but some work indicates that the system is colloidal (*24*, *47*, *59*). By action of ammonium molybdate upon molybdenum chloride Berzelius (*10*) obtained a blue colored solid to which he gave the formula $MoO_2.4MoO_3.xH_2O$. For solid molybdenum blue Ephraim (*24*) accepts this formula, but Latimer and Hildebrand (*40*) prefer that of Muthmann (*48*) and call the compound molybdenyl molybdate, $(MoO_2)_2MoO_4$. The reduction is affected by light, a method of measuring the intensity of ultraviolet radiation being based upon the rate of formation of the blue color by a sodium molybdate solution acidified with hydrochloric and formic acids (*60*).

The reduction product of heteropoly compounds is also uncertain. Two recent authors claim that the phosphorus in molybdiphosphates acts only as a catalyst (*8*). Older work indicates (*17*, *20*, *22*) that the formula for the blue material separated may be expressed as $H_3XO_4(4MoO_3.MoO_2)_2$, in which X is phosphorus or arsenic. Others believe there is an equilibrium (*35*) such as

$$\text{Molybdiphosphoric acid} + \text{chlorostannite} \rightleftharpoons \text{molybdenum blue} + \text{chlorostannate}$$

From spectrophotometric data Gripenberg (*34*) assumed that six of the twelve molybdenum atoms in molybdiphosphate are reduced. On reduction with molybdenum chloride the final product is said to be $H_3PO_4(Mo_6O_{17}).xH_2O$, in which x is about 19 (*4*). Apparently a definite stage is reached in the reduction, as such a reaction is the basis of titrimetric methods for determining copper, iron, magnesium, and zinc (*30*). In a similar procedure for phosphate the reduced blue material, stated to be $H_3PO_4(11MoO_3.MoO_2)_2$, is titrated with permanganate (*13*).

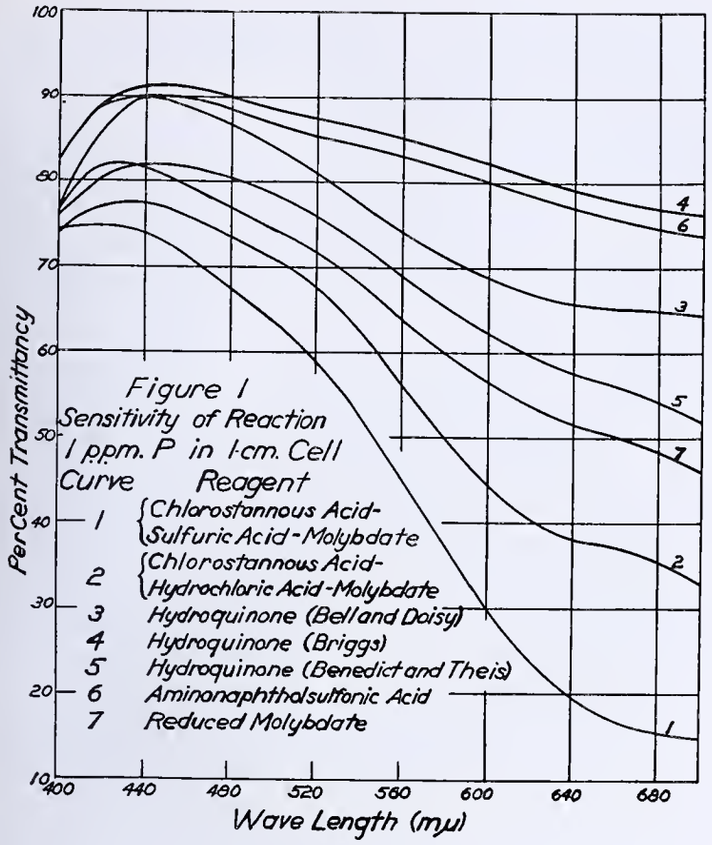
COLORIMETRIC APPLICATIONS. Since molybdenum blue arises from an interaction of oxidizing and reducing systems, there is a possibility of determining either reductants, through their action on some heteropoly compound, or heteropoly oxidants, through their action on suitable reductants. Thus, the cerous ion, in strongly basic solution, reduces molybdiphosphate (*38*); similarly, ascorbic acid may be determined as a reductant (*70*).

Several comparative visual or filter-photometric studies have been made on reductants as such (*1*, *3*, *35*). Specific compounds include chlorostannous acid (*50*), aminonaphtholsulfonic acids (*28*), benzidine (*26*), gallic acid (*52*), hydriodic acid (*72*), hydroquinone (*6*), *p*-methylaminophenol (*43*), sodium thiosulfate (*45*), and a molybdate reduced with some metal, usually molybdenum itself (*73*).

The determination of the central atom in heteropoly compounds has found most use.

Determination of Phosphorus. Most work has been done with phosphorus, presumably because of its wide distribution and biological importance. The principal points to be noted are the variety of reductants and the variations in their use.

Denigès (*19*) used chlorostannous acid under conditions to reduce only the heteropoly acid. Truog and Meyer (*64*) studied the effect of aluminum, calcium, iron, magnesium, manganese, and nitrate on the color, in addition to modifying the reagent and providing for interference of silica. Dickman and Bray (*23*) recommended hydrochloric acid in the reagent. Color studies have been made with the Leitz photometer (*5*, *54*) and the Lovibond tintometer (*36*). Color standards include indigo carmine (*29*), a reduced molybdate (*46*), and two mixtures of copper and



cobalt salts (*18*). Applications include the determination of phosphorus in argillaceous material (*68*), fertilizers (*55*), plants (*56*), soils (*16*), water (*25*), and tissues and blood (*11, 39*).

Bell and Doisy recommended hydroquinone as a reductant (*6*). Briggs (*14*) modified the reagent by making it acidic instead of alkaline, while Benedict and Theis (*7*) developed the color by heating. Denigès (*21*) and Rimington (*58*) pointed out certain errors in the method. This modification has been applied to blood (*57*), milk (*53*), soil extract (*2*), sugar (*15*), urine (*66*), and water (*61*).

Fiske and Subbarrow (*28*) used aminonaphtholsulfonic acid as a reductant. The best isomer has the amino and hydroxy groups para to each other (*65, 69*). Applications include orthophosphate in meta- and pyrophosphates (*12*) and phosphorus in muscle extract (*44*).

Zinzadze (*74, 76*) used a molybdate reduced with metallic molybdenum as reductant. By addition of bisulfite this reagent may be used to determine phosphorus in soils (*71*) and urine (*33*), in the presence of arsenates, iron, nitrates, and silicates. Copper (*21*) or a mixture of copper and lead (*42*) may be used in preparing the reagent.

Feigl (*26*) used benzidine in ammoniacal solution as a reductant. Tartaric acid prevents interference of arsenates (*27*). In this way phosphorus has been determined in rocks (*41*) and in water (*63*).

Determination of Silicon. By reduction of a molybdisilicate Isaacs (*37*) and Urbach (*67*) determined silicon. The method was criticized by Bertrand (*9*) but Foulger (*31*) refuted the criticism. Reducing agents include chlorostannous acid (*49*), hydroxylamine (*51*), sodium sulfite, sodium hyposulfite, and hydroquinone (*62*).

Determination of Arsenic. Zinzadze (*75*) and Truog and Meyer (*64*) determined arsenic by reducing the heteropoly molybdiarsenate. The reductants were reduced molybdate and chlorostannous acid, respectively.

Experimental Work

All color measurements were made with a General Electric photoelectric spectrophotometer, the absorption cells being 1.000 cm. thick and the spectral band 10 mμ wide. Distilled water was used in the blank cell, since the blank solution itself was slightly colored and the over-all color was desired.

Determination of Phosphorus. A standard phosphate solution containing 0.1 mg. of phosphorus per ml. was prepared by dissolving 0.4390 gram of recrystallized potassium dihydrogen phosphate in water, adding 15 ml. of 6 *N* sulfuric acid, and diluting to 1 liter. Dilutions of this stock solution were used.

Reduction with Chlorostannous Acid. A standard solution of chlorostannous acid reductant was prepared by dissolving about 100 grams of "stannous chloride" crystals in 100 ml. of concentrated hydrochloric acid and diluting to 4 liters. The solution was protected from the air by a layer of "Finol" 1 cm. thick, and the entrance to the bottle was trapped twice with alkaline pyrogallol. The original air in the bottle was removed by bubbling natural gas through the solution. The solution, found to be 0.1044 *M*, was oxidized about 2 per cent at the end of 14 weeks.

To prepare the reduced heteropoly compound, 10 ml. of a solution containing 0.01 mg. of phosphorus per ml. were measured into a 100-ml. volumetric flask and then 4 ml. of 2.5 per cent ammonium molybdate in 10 *N* sulfuric acid were added. After diluting to about 40 ml., 0.50 ml. of 0.1 *M* chlorostannous acid was added, and the solution was diluted to the mark. At the end of 7 minutes the spectrophotometric curve was determined. Spectral transmission curves, determined under different conditions for 1 p. p. m. of phosphorus, are shown in Figure 1.

The relative amounts of acid and molybdate in the reagent are important since, if the acid/molybdate ratio is too low, some of the molybdate is reduced along with the heteropoly acid; if too high, there is a marked decrease in the intensity of the color. The optimum ratio is that in which a blank would give no blue color. Tests showed that a 2.5 per cent solution of ammonium molybdate in 10 *N* sulfuric acid, or one 1.5 per cent in ammonium molybdate in 3.5 *N* hydrochloric acid, is the best reagent. Sufficient reagent should be used to make the final acidity about 0.4 *N* in sulfuric acid or 0.7 *N* in hydrochloric acid. Any residual acidity in the solution to be measured is neutralized to a slight yellow, using α-dinitrophenol as indicator, before adding the molybdate reagent. The amount of reductant need not be so carefully controlled as that of the molybdate reagent. Enough should be added to react with all the heteropoly acid, but too much leads to turbidity on standing. About 0.5 ml. of 0.1 *M* solution is sufficient for 100 ml.

Using 1-cm. cells, the range of concentration is 0.05 to 2.5 p. p. m. of phosphorus. A graph of the transmittancy on a logarithmic scale against concentration shows that the ammonium molybdate–sulfuric acid solutions conform to Beer's law up to 1 p. p. m., above which there is a negative deviation. The hydrochloric acid solutions conform up to 2.5 p. p. m.

Time of standing must be rather closely controlled. The full color develops in 3 to 4 minutes and begins to fade in about 10 minutes. The color measurement must be made, therefore, within this interval.

In studying the effect of diverse ions the ammonium molybdate–sulfuric acid reagent was used. The general procedure was the same as already described, except for the addition of the solution containing the diverse ion immediately preceding the ammonium molybdate reagent.

Of the 31 cations studied, aluminum, ammonium, beryllium, calcium, cerous, lithium, magnesium, manganese, potassium, sodium, and thorium in concentrations of 500 p. p. m. cause an error of less than 2 per cent of the phosphorus present. Barium, lead, mercuric, mercurous, silver, strontium, and zirconyl ions interfere by giving precipitates. Antimonous, bismuthyl, cadmium, chromic, cupric, ferric, and zinc ions form soluble complexes and thus bleach the color. Ferrous ions interfere, possibly through oxidation to the ferric state. Ceric ions interfere by oxidation. Cobaltous, nickelous, and uranyl ions interfere with their own hue. A summary of the effects of these cations is given in Table I.

Of the 32 anions studied acetate, benzoate, carbonate, formate, lactate, nitrate, perchlorate, salicylate, sulfate, sulfite, and tetraborate ions do not interfere in concentrations of 500 p. p. m. Oxidizing or reducing ions which interfere include chlorate, chlorostannate, cyanide, dichromate, nitrite, thiocyanate, thiosulfate, and vanadate. All the halogens interfere, the effect of fluoride being greatest. They decrease the intensity of the color, as do citrate, oxalate, and tartrate. Tungstate is reduced to form tungsten blue and must be absent. Arsenate, pyrophosphate, and silicate form heteropoly acids, which may be reduced and thus must be absent. Ar-

Table I. Effect of Diverse Ions

Ion	Added as	Amount	Error	Permissible Amount[a]
		P. p. m.	%	*P. p. m.*
Ag^+	$AgNO_3$	..	Ppts.	0
AsO_3^{---}	$NaAsO_2$	10	13	1
AsO_4^{---}	Na_3AsO_4	1	25	0
Ba^{++}	$Ba(NO_3)_2$	..	Ppts.	0
Bi^{+++}	$Bi(NO_3)_3$	50	8	10
Br^-	NaBr	500	4	250
Cd^{++}	$Cd(NO_3)_2$	500	2	500
Ce^{++++}	$Ce(SO_4)_2$	100	22	10
Cl^-	KCl	500	4	250
ClO_3^-	$KClO_3$	100	11	10
Co^{++}	$Co(NO_3)_2$	500	8	50
Cr^{+++}	$Cr_2(SO_4)_3$	25	New hue	25
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	10 Cr	5	0
CN^-	KCN	100	0	100
Cu^{++}	$Cu(NO_3)_2$	25	14	3
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	25	8	5
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	100	2	100
$C_6H_5O_7^{---}$	$H_3C_6H_5O_7$	100	5	30
F^-	NaF	25	8	5
Fe^{++}	$FeSO_4.(NH_4)_2SO_4$	100	2	100
Fe^{+++}	$Fe(NO_3)_3$	25	2	10
Hg^+	$HgNO_3$	..	Ppts.	0
Hg^{++}	$HgCl_2$	..	Ppts.	0
I^-	KI	500	12	100
MoO_4^{--}	$(NH_4)_2MoO_4$	100 Mo	17	0
Ni^{++}	$Ni(NO_3)_2$	100	New hue	100
Pb^{++}	$Pb(NO_3)_2$	..	Ppts.	0
$P_2O_7^{--}$	$Na_4P_2O_7$	5	9	0
Sb^{+++}	$SbCl_3$	50	27	5
SCN^-	KSCN	100	4	50
$S_2O_3^{--}$	$Na_2S_2O_3$	25	4	10
SiO_3^{--}	Na_2SiO_3	50 Si	10	10
$SnCl_6^{--}$	H_2SnCl_6	25 Sn	22	0
Sr^{++}	$Sr(NO_3)_2$	50	Ppts.	50
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	500	2	500
VO_3^-	$NaVO_3$	10	0	10
WO_4^{--}	Na_2WO_4	100	New hue	100
Zn^{++}	$Zn(NO_3)_2$	500	2	500
ZrO^{++}	$ZrO(NO_3)_2$	..	Ppts.	0

[a] To give error within 2 per cent phosphorus.

senite interferes, possibly by oxidation to arsenate. Table I summarizes these effects.

Typical curves for the reduced molybdate (*46*) and indigo carmine (*29*) color standards are shown in Figure 2. Visually these standards look somewhat like the reduced heteropoly solutions, but there is no spectrophotometric match. In addition, the reduced molybdate solutions are not much more stable than the solutions to be measured. The copper standards (*18*) did not even resemble visually the solutions they are supposed to match.

Reduction with Hydroquinone. In the use of hydroquinone as a reductant three variations in procedure were followed.

1. Bell and Doisy Method. In this procedure the appropriate amount of phosphate solution, usually 0.25 mg. of phosphorus, was transferred to a 100-ml. flask. To this were added 5 ml. of a 5 per cent molybdate solution (in 1 *N* sulfuric acid) and 5 ml. of 2 per cent hydroquinone (in 0.03 *N* sulfuric acid), and the solution allowed to stand 5 minutes. Then 25 ml. of sulfite solution (200 grams of sodium carbonate plus 37.5 grams of sodium sulfite per liter) were added, the solution was diluted to the mark, and the color was measured immediately.

Since increased basicity makes the solution more blue and also increases the intensity of the color, the amount of sulfite solution must be controlled rather closely. Small changes in the concentrations of molybdate and hydroquinone do not greatly affect the color. There is little change in color on allowing the solution to stand 1 to 25 minutes after adding the hydroquinone, providing the measurement is made soon after addition of the sulfite reagent. The color begins to fade as soon as the sulfite is added, but this is not serious within 10 to 15 minutes. The range of concentration in a 1-cm. cell is 0.1 to 10 p. p. m. of phosphorus, and the system conforms to Beer's law over this range.

2. Briggs Method. To an appropriate amount of phosphate solution in a 100-ml. flask there were added 5 ml. of molybdate reagent (25 grams of ammonium molybdate in 300 ml. of water plus 200 ml. of a solution containing 75 ml. of concentrated sulfuric acid), 4 ml. of hydroquinone (0.5 per cent solution in 0.01 *N* sulfuric acid), and 1 ml. of 20 per cent sodium sulfite solution. After diluting to the mark and allowing to stand for 30 minutes, the color was measured.

The color depends somewhat upon the amount of reagents used but more upon the time of standing. Since the reaction is incomplete even after 5 days, the color measurement must be made at some definite time after preparing the solution. The applicable range is 0.5 to 12 p. p. m. of phosphorus and the system conforms to Beer's law for this range.

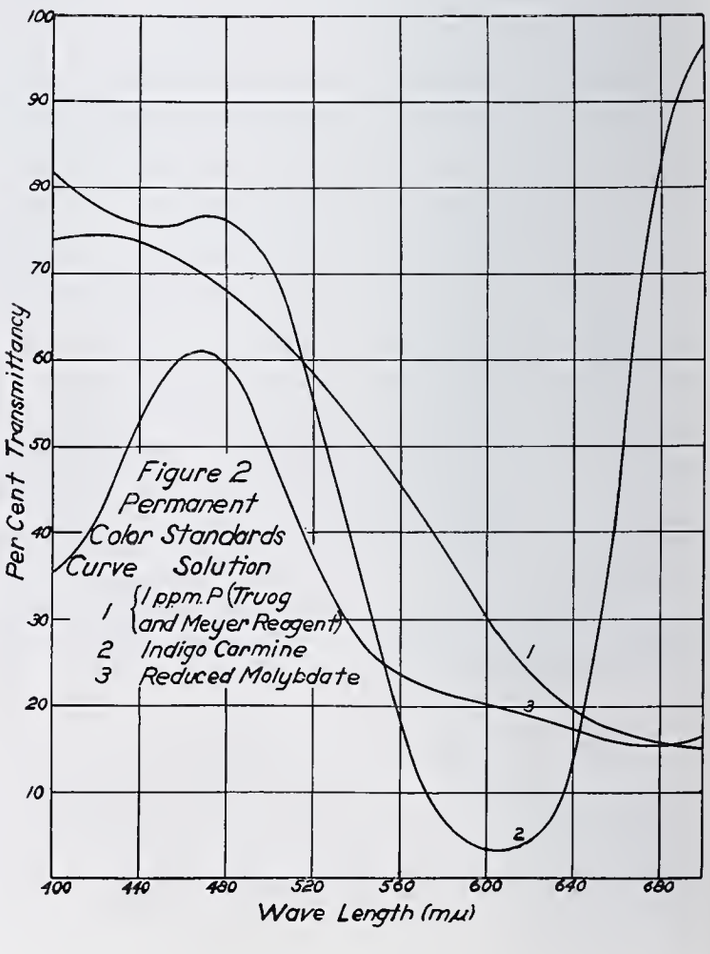
3. Benedict and Theis Method. To an appropriate amount of phosphate solution in a 50-ml. flask there were added 5 ml. of molybdate reagent (10 grams of molybdic anhydride in 25 ml. of 20 per cent sodium hydroxide diluted to 250 ml. plus an equal volume of concentrated sulfuric acid) and 5 ml. of hydroquinone (0.5 per cent in 15 per cent sodium bisulfite), and the solution was diluted to about 40 ml. After heating at 100° for 25 minutes, the solution was cooled and diluted to the mark, and the color was measured.

Although the color will develop slowly at room temperature, heating for 20 to 25 minutes gives a reproducible system. The color in the heated solution will increase about 4 per cent on standing 24 hours. The range of concentration is 0.1 to 5 p. p. m. and the system conforms to Beer's law up to 3 p. p. m.

Reduction with Aminonaphtholsulfonic Acid. To an appropriate amount of phosphate solution in a 50-ml. flask 10 ml. of molybdate reagent (2.5 per cent ammonium molybdate in 5 *N* sulfuric acid) were added, and the solution was diluted to 35 to 40 ml. After adding 5 ml. of reductant (0.5 gram of aminonaphtholsulfonic acid plus 195 ml. of 15 per cent sodium bisulfite plus 5 ml. of 20 per cent sodium sulfite, all diluted to 250 ml.), the flask was diluted to the mark and allowed to stand 30 minutes before measurement.

Of the several isomeric aminonaphtholsulfonic acids, the 1,2,4, 2,5,7, 1,4,8, and 2,6,8 compounds were used. The first proved to be the most effective. Increasing the amount of reductant increases the depth of color, but the system is least sensitive to this variable with the 1,2,4 isomer.

Since it was impossible at any elevated temperature to reduce all of the heteropoly acid without some of the uncombined molybdate, heating was impractical. Therefore the color was measured 30 minutes after the solutions were prepared. Low acidity leads to reduction of the molybdate and too much acid causes the color to develop more slowly. Over the range 0.6 to 1.3 *N* there is little difference in color if the solution is allowed to stand for only 30 minutes. The amount of molybdate reagent produces little effect on the color as long as there is sufficient to react. The

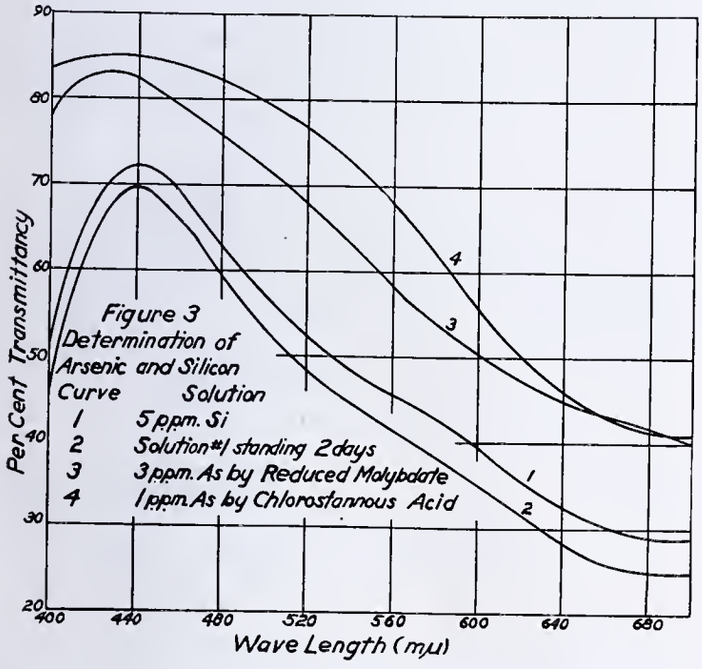


range of concentration is 0.2 to 10 p. p. m. of phosphorus for which Beer's law applies.

Reduction with Reduced Molybdate. To make the reductant, 20.06 grams of molybdic anhydride were dissolved in 505 ml. of 25 *N* sulfuric acid by heating, and the solution was cooled and diluted to 500 ml. with water. Half of this solution was boiled 15 minutes with 0.89 gram of molybdenum, cooled, and diluted to 250 ml. This solution was standardized against permanganate and then mixed with the original solution to make the final solution 0.1 *N* as a reductant. It was stored in a brown glass bottle and diluted 10 times before using.

To the phosphate solution in a 50-ml. flask there were added 5 ml. of 0.1 *N* sulfuric acid and 5 ml. of 8 per cent sodium bisulfite, and the solution was diluted to about 35 ml. This was allowed to stand overnight or for an hour at 100° to reduce any arsenic or iron present. To it were then added 5 ml. of molybdate reagent and the solution was heated for 30 minutes at 100°. After cooling and diluting to the mark, the color was measured.

The full color develops in 25 minutes at 100° and continued heating for an hour has little effect. The acidity must be rather carefully controlled, as higher values decrease the color intensity. Usually 5 ml. of *N* sulfuric acid are added to prevent the formation of molybdisilicic acid which would give high results if silica were present. Provided enough is added to react with the phosphorus excess molybdate has little effect. Small amounts of bisulfite have little effect, but large amounts increase the depth of color, possibly by increasing the pH and causing some reduction of uncombined molybdate. The range of concentration is 0.1 to 5 p. p. m. of phosphorus. The system conforms to Beer's law up to 4 p. p. m. and then deviates sharply from it. There was an error of about 5 per cent due to fading on standing 22 hours. A study of ferric iron, arsenate, arsenite, silicate, and tetraborate showed that the first four interfere and should not exceed 2, 25, 100, and 25 p. p. m., respectively.



Determination of Silicon. Since molybdates may react with silicates to form molybdisilicic acid, the molybdenum blue reaction can be used to determine silicon.

The standard silica solution was made by dissolving 1.255 grams of sodium silicate monahydrate, $Na_2SiO_3.9H_2O$, in water containing 1 gram of sodium hydroxide and diluting to 250 ml. This solution, containing 1 mg. of silica per ml., was diluted tenfold for use.

To an appropriate amount of this silicate solution in a 50-ml. flask there were added 20 ml. of water, 6 ml. of 10 per cent acetic acid, and 10 ml. of 10 per cent ammonium molybdate. The solution was heated on a boiling water bath for 5 minutes, and 4 ml. of saturated sodium sulfite solution were added, after which the solution was cooled, diluted to the mark, and measured.

Table II. Reductants for Determining Phosphorus

Item Compared	Chlorostannous Acid	1-Amino-2-naphthol-4-sulfonic Acid	Reduced Molybdate
P concn. at 50% transmission[a], p. p. m. phosphorus	0.75	2.21	0.90
Working range, 1-cm. cell, p. p. m. phosphorus	0.02–2.0	0.2–10	0.1–5
Time and temperature, to develop color	3 min., 20–25°	30 min., 20–25°	30 min., 100°
Stability	15 min.	Changes[b]	10 hours
Effect of excess reagent	Slight decrease	None	Slight decrease
Effect of excess reductant	Slight increase	Slight increase	Slight increase
Conformity to Beer's law, p. p. m. phosphorus	0–1	0–10	0–4

	Hydroquinone		
	Bell and Doisy	Briggs	Benedict and Theis
P concn. at 50% transmission[a], p. p. m. phosphorus	1.65	2.62	1.06
Working range, 1-cm. cell, p. p. m. phosphorus	0.1–10	0.1–15	0.1–5
Time and temperature to develop color	5 min., 20–25°	30 min., 20–25°	25 min., 100°
Stability	15 min.	Changes[b]	10 hours
Effect of excess reagent	Slight decrease	.....	.....
Effect of excess reductant	Slight increase	Increase	.....
Conformity to Beer's law, p. p. m. phosphorus	0–10	0–12	0–3

[a] For 1-cm. cell at 700 mu.
[b] Measurement must be made after definite time.

The range of concentration is somewhat higher than for phosphorus, being 1 to 15 p. p. m. of silicon. If the color is measured a short time after preparing the solutions, they do not conform to Beer's law; but if they stand 2 days, the law applies. At high concentrations of silica the reduction seems to proceed more slowly than at lower values. A typical curve for the blue system is shown in Figure 3.

Determination of Arsenic. A heteropoly molybdiarsenate, formed from molybdate and arsenate, gives the molybdenum blue reaction and thus may serve for the determination of arsenic.

A standard arsenic solution was prepared by dissolving 0.950 gram of arsenic acid in water to which were added 100 ml. of *N* sulfuric acid and dilute permanganate to make the solution just pink. This solution, diluted to 1 liter, contains 0.5 mg. of arsenic per ml. It was diluted tenfold for use.

Using the reduced molybdate solution as reductant, the same procedure was followed in preparing the blue systems as for the determination of phosphorus, except that the addition of acid and bisulfite was omitted. The system conforms to Beer's law up to 8 p. p. m. of arsenic and then deviates sharply from it. The solutions are much more stable than the corresponding phosphorus solutions. On standing 5 days those containing less than 8 p. p. m. did not fade, but those of higher concentration changed appreciably. The chlorostannous acid reagent was tried also, using the same procedure as for phosphorus. This system conforms to Beer's law up to 2 p. p. m. Turbidity developed on standing. Selected curves are shown in Figure 3.

Discussion

Since the molybdenum blue method is based upon the selective reduction of the molybdenum in a heteropoly acid in presence of excess molybdate, there are several variables which must be rather closely controlled. These include ratio of molybdate to acid, acidity, amount of molybdate, time to develop the color, temperature at which the color develops, and the presence of certain diverse ions in the solution.

The optimum ratio of molybdate to acid is that which, in a given time, would give maximum reduction of the heteropoly

acid with no reduction of the uncombined molybdate. This ratio, once established, should be kept constant. A convenient means is to prepare the molybdate reagent in a given concentration of acid. Ordinarily the amount of reagent and of reductant will not greatly affect the color, provided, of course, there is sufficient present to effect the desired reaction. A general study of the effect of diverse ions, made with solutions using chlorostannous acid as reductant, showed many interferences.

A comparison of some of the characteristics of the different modifications for determining phosphorus, shown in Table II, indicates that the chlorostannous acid procedure is the most sensitive and requires the least time to prepare the color for measurement. The main disadvantages are the tendency to develop turbidity, especially with low concentrations of phosphorus, the relative instability of the color, and the difficulty of preserving a chlorostannous acid solution. The reduced molybdate reagent rivals it in sensitivity, conforms to Beer's law up to 4 p. p. m. of phosphorus, has greater stability, and excess reagent has very little effect. Its main disadvantage is the necessity of heating 30 minutes at 100° to develop the color. The reagent itself must be carefully prepared, but then it is reported to be stable for years. Silicate and arsenate interfere less than in the chlorostannous acid method. Hydroquinone and aminonaphtholsulfonic acid seem to have no advantages over the other two reductants. They are less sensitive and require time to develop the color, and in some cases a definite temperature must be maintained for a definite time.

Apparently each process of reducing the heteropoly acid to molybdenum blue produces a system of somewhat different light-absorptive characteristics. Thus in Figure 1 the spectral transmission curves are all for the same concentration of phosphorus but different reductants. It is obvious that no single permanent standard would serve. In addition, Figure 2 shows the failure of the proposed standards to match the system for which they were proposed.

Figure 3 shows curves for the application of the method to the determination of arsenic and silicon. Solutions for arsenic have nearly the same color as those for phosphorus. The reduced molybdate-arsenate solutions are more stable than the corresponding phosphorus compounds. In using acetic acid and sodium sulfite to reduce the silicon complex the process proceeds slowly with high concentrations of silicon. Freshly prepared solutions deviate markedly from Beer's law, but after two days the divergence nearly disappears.

Summary

A spectrophotometric study has been made of the application of the molybdenum blue method to the colorimetric determination of phosphorus, arsenic, and silicon. This included the range of the methods, conformity of the blue system to Beer's law, and the effects of variables such as time, temperature, acidity, and amounts of reactants upon the color developed. In addition, there was determined for phosphorus the effect of 63 diverse ions, the relative merits of chlorostannous acid, hydroquinone, aminonaphtholsulfonic acid, and a reduced molybdate solution as reductants, and the inadequacy of several proposed permanent standards.

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Ultraviolet Absorption Spectra of Linseed Oil

Determination of Bodied-*in-Vacuo* and Blown Linseed Oil in Mixtures with Raw Linseed Oil

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An examination of the ultraviolet absorption spectra of a series of linseed oils bodied by blowing at 220° F. and by heating *in vacuo* at 585° F. shows that the absorption spectrum of a mixture of either type of oil with raw linseed oil may be used for the analysis of the bodied constituent.

Both types have maxima at 2320 Å., but the presence of a band with a maximum near 2700 Å. in the oxidized series distinguishes these oils from the oils bodied *in vacuo*. In the bodied-*in-vacuo* series the band at 2320 Å. has been attributed to the formation of conjugated octadecadienates as a step in the polymerization process.

Making use of the 2700 Å. band for the qualitative examinations and of the 2320 Å. maximum for the quantitative determinations, the amount and kind of bodied oil in mixtures with raw linseed oil have been measured. The amounts of bodied oil ranged from 5 to 50 per cent, and the analyses were made with an average percentage error of ±2.12.

In addition to absorption spectra, iodine number, refractive index, acid number, specific gravity, color, and viscosity are given.

THE ultraviolet absorption spectra have been used by various investigators (*1*, *2*, *8*, *12*, *16*) in studies of the drying oils and their fatty acids. Bradley and Richardson (*2*) and Bradley and Johnston (*1*) have recently used this technique in connection with their investigations of the changes accompanying the polymerization by heat treatment of oils. Conjugation, as pointed out by Crymble *et al.* in 1911 (*5*) may be detected by the ultraviolet absorption spectra, and by this means a much more reliable estimate of this type of unsaturation may be obtained than by using the iodine value.

This article points out the differences in the absorption spectra of blown, or oxidized, linseed oil and of oil polymerized *in vacuo*, as well as the use to which the ultraviolet absorption spectra may be put in the qualitative and quantitative determination of the amounts of these oils which have been added to untreated, or raw, linseed oil.

Instrument and Methods

All the absorption data were obtained from measurements made with a photoelectric spectrophotometer (*11*) employing a Hilger double monochromator with crystal quartz optics. As a source of ultraviolet light, a Munch (*18*) hydrogen discharge tube with a fused quartz window was used. Solutions of the oils for the curves presented in Figure 1 were made in diethyl ether, purified for optical use (*4*), and dilutions were made, if necessary, to maintain the values of $\log_{10}\frac{I_0}{I}$ between 0.200 and 0.800. Absorption cells 1 cm. in length with matched crystalline quartz end plates were used.

Slit widths of 0.25 mm. were used at 2320 Å. The spectral region isolated (*11*) at this wave length is 5.4 Å. for a slit width of 0.25 mm. However, when slit widths of 0.20 to 1.0 mm. were used at this wave length with the same solution the value of $\log_{10}\frac{I_0}{I}$ remained constant.

At 2680 Å. a slit width of 0.15 mm., isolating a region of 5.6 Å., was used. A constant value for $\log_{10}\frac{I_0}{I}$ was obtained at this wave length for the same solution when slit widths of 0.12 to 0.60 mm. were used.

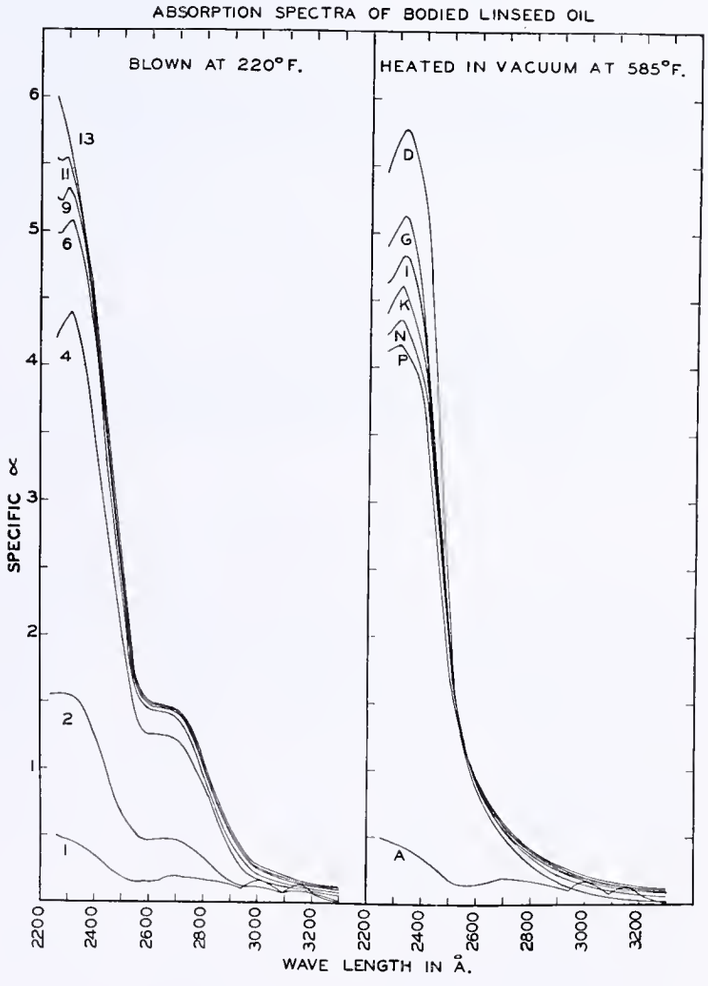

FIGURE 1

The effectiveness of the monochromator in the elimination of stray light at these wave lengths is shown by the fact that with the slits open to 1.0 mm., a 72-watt Mazda lamp, used as a light source in the visible spectrum, caused no galvanometer deflection. In the visible region with this same source, slits approximately 0.06 mm. wide are customarily used.

Lambert's law was demonstrated for the heat-bodied oils when cell lengths of 1 and 4 cm. were used.

The absorption curves are plotted, using the value of the specific absorption coefficient on the ordinate and the wave length in Ångstrom units on the abscissa.

Table I. Linseed Oil Bodied in Monel at 585° F.

Designation	Acid No.	Specific Gravity	Iodine No.	Refractive Index	Color[a] (20 Mm.)	Viscosity *Poises*
A	0.25	0.9310	178.2	1.47855	88–63–73	0.40
D	3.85	0.9510	139.3	1.48412	80–56–67	3.82
G	5.36	0.9608	131.2	1.48681	78–40–57	13.8
I	5.76	0.9652	128.5	1.48793	63–29–48	25.5
K	6.28	0.9664	127.5	1.48888	56–20–41	45.3
N	6.85	0.9688	126.4	1.48971	47–13–28	80.0
P	6.94	0.9703	126.1	1.48986	43–11–26	101.

[a] Color readings were made with Hess-Ives machine, first figure being reading of light transmission through a 20-mm. cell and green filter. Second is blue-violet reading, and third the blue.

Table II. Linseed Oil Blown in 100-Barrel Iron Tank at 220° F.

Designation	Acid No.	Specific Gravity	Iodine No.	Refractive Index	Color[a] (20 Mm.)	Viscosity *Poises*
1	0.24	0.9311	180.3	1.47858	83–64–72	0.40
2	0.92	0.9364	173.4	1.47914	88–72–87	0.60
4	2.87	0.9640	145.7	1.48221	89–54–66	2.25
6	3.67	0.9740	136.2	1.48341	84–46–74	4.45
9	4.31	0.9848	127.0	1.48453	78–34–57	10.4
11	4.57	0.9898	123.2	1.48517	76–24–50	16.8
13	4.59	0.9937	120.5	1.48563	73–16–42	24.5

[a] Color readings were made with Hess-Ives machine, first figure being reading of light transmission through a 20-mm. cell and a green filter. Second is blue-violet reading, and third the blue.

$$\text{Specific } \alpha = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

α = absorption coefficient
I_0 = intensity of radiation transmitted by the solvent
I = intensity of radiation transmitted by the solution
c = concentration of solute in grams per 1000 ml.
l = length in centimeters of solution through which the light passes

Discussion of Absorption Curves

Untreated linseed oil has very low absorption for light in the ultraviolet region of wave length longer than 2300 Å., the specific alpha at 2320 Å. being only 0.34 for the sample of raw oil used in making up the mixtures for analysis. It has been shown (*12*, *19*) that neither the fatty acids nor their esters and glycerides, unless conjugated, cause specific absorption in the range of wave lengths with which this study is concerned. There is some evidence (*1*) that cyclization causes a general increase in the specific absorption value in the ultraviolet region, and for this reason not too much significance can be attached to estimates of the actual amounts of conjugated materials present in bodied oils.

Conjugation is of great importance in causing absorption in this region, illustrated by the high specific absorption at 2700 Å. found by Dingwall and Thomson (*8*) for α-eleostearic acid and the value of 120 found for 9,11-linoleic acid in the 2300 Å. region by Hulst (*12*). In this latter region 2,3-oleic acid, in which the double bond is conjugated with the carboxyl, also shows strong absorption, although no maximum could be observed by Hulst (*12*). Working with more simple compounds, Hausser (*10*) found the maxima for crotonaldehyde and crotonic acid in hexane to be, respectively, at 212 and 208 millimicrons, the double bond in crotonaldehyde being conjugated with an oxygen atom of the aldehyde group and in crotonic acid with that of the carboxyl group.

With both the blown and the heat-bodied oils (Figure 1) the development of a relatively high absorption band with a maximum between 2320 and 2325 Å. is characteristic. This band, in the case of esters and oils bodied in the absence of oxygen, has been attributed by Bradley (*2*) to the formation of conjugated octadecadienates by isomerization of the double bonds as a step in the polymerization process. This evidence is in support of Brod *et al.* (*3*) and of Scheiber (*20*) who presented similar hypotheses. Increased absorption of ultraviolet radiation in connection with prolonged heating on saponification has also been shown to occur (*6*, *7*, *9*, *13*, *17*). In this connection, the absence of any absorption band in the region of 2700 Å. in the bodied-*in-vacuo* oils must be taken as evidence that no systems of three conjugated double bonds are present. Kino (*14*) found that the polymerization of methyl linolenate takes place without a shift of the double linkages.

The oils bodied *in vacuo* and those bodied by blowing with air show, at first, a very rapid fall in the iodine number with very little corresponding increase in the viscosity (Tables I and II). In the heat-bodied series the iodine number drops from 178.2 for sample A, viscosity 0.40 poise, to 139.2 for sample D, viscosity 3.82. As the heat treatment is continued the viscosity rises rapidly without a comparable decrease in the iodine number.

The period during which the iodine number falls rapidly is also that in which the acid number, specific gravity, and refractive index rise sharply. Several investigators have noted that the first changes induced by the heat treatment seem to be due to intramolecular rather than to intermolecular reactions. During this period the marked change in the absorption spectrum is also noted. When the viscosity has reached 3.85 poises the specific alpha for the heat-bodied oil has a value of about 5.7 at 2320 Å. compared to 0.34 for the original oil. With further heating, however, the specific absorption at 2320 Å. falls gradually with the rise in viscosity and the fall in the iodine number (Figures 2 and 3).

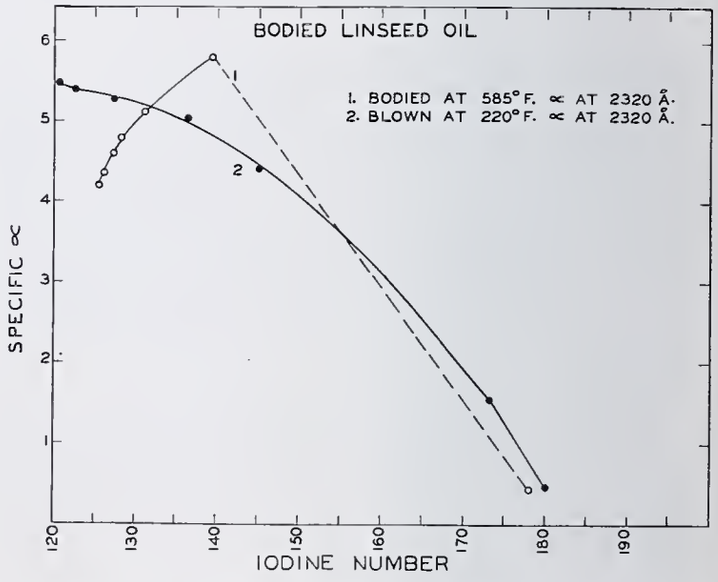

Figure 2. Comparison of Specific Absorption Coefficients at 2320 Å. with Iodine Numbers of Oxidized and Heat-Bodied Linseed Oils

Based on the specific absorption coefficient of 120 found by Hulst (*12*) for 9,11-linoleic acid and neglecting other possible sources of absorption, the conjugated linoleic acid is estimated to be present to the extent of about 4.7 per cent in sample D, specific alpha 5.7, and about 3.5 per cent in sample P, specific alpha 4.2.

In the blown oils, however, the specific absorption coefficient rises gradually with the viscosity (Figure 3). This rise continues and in sample 13, viscosity 24.5 poises, the specific

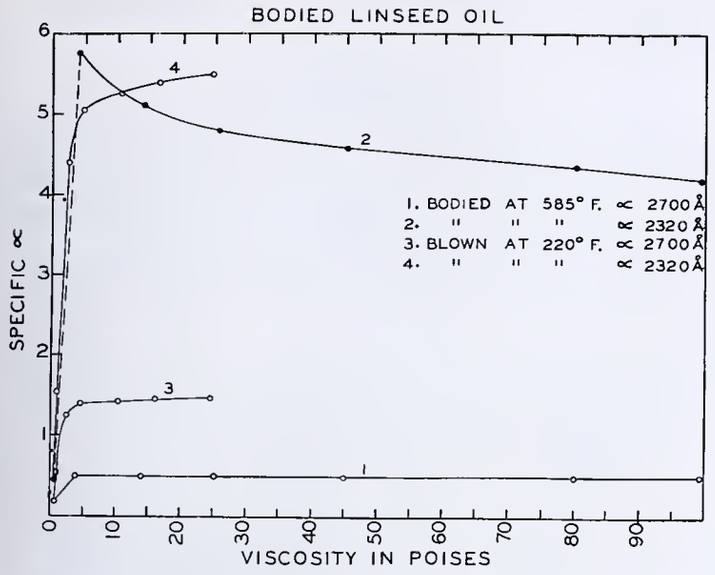

FIGURE 3. COMPARISON OF SPECIFIC ABSORPTION COEFFICIENTS AT 2320 Å. AND 2700 Å. WITH VISCOSITY OF OXIDIZED AND HEAT-BODIED LINSEED OILS

alpha is 5.7. In this sample no definite maximum was observed.

The small bands at 3000 and 3160 Å., present in the original oil, disappear very early during either type of treatment.

When the specific alpha at 2320 Å. is plotted against the iodine number (Figure 2), another difference between the two types of oils is apparent. In the oxidized series the specific alpha continues to increase with decreasing iodine value, while in the heat-bodied series the specific alpha first increases sharply and then falls with the decreasing iodine values.

TABLE III. SPECIFIC ABSORPTION AT 2700 Å. OF BLOWN LINSEED OILS

Sample	Viscosity *Poises*	Specific α at 2700 Å.	Deviation
6	4.45	1.39	−0.04
9	10.4	1.43	0.00
11	16.8	1.44	+0.01
13	24.5	1.45	+0.02
	Av.	1.43	±0.017
	Raw linseed	0.057	

Use of Absorption Curves for Analysis

The distinguishing characteristic between the spectra of the oxidized and the bodied-*in-vacuo* oils is the appearance of a second band between 2600 and 2700 Å. in the oxidized series. This band (Figure 1) continues to increase in height until a viscosity of about 4.45 poises and an iodine number of 136 is reached. Blowing continued after this condition is obtained produces no appreciable change in the specific alpha between these wave lengths, although the viscosity continues to increase. Table III, showing the average value of the specific alpha of blown samples having a viscosity of 4.45 poises and above to be 1.43 ± 0.017 at 2700 Å., illustrates this fact. Thus it is possible to use the value 1.43 at 2700 Å. to determine the amount of blown linseed oil of this series, from a viscosity of 4.5 to 25 poises, in a mixture with raw linseed oil.

The specific absorption coefficients of the bands in the oxidized series remain in the same relative order at 2700 as at 2320 Å. It is not possible, in the light of the present investigations, to identify the oxidized product which causes this band.

In the heat-bodied series the relative order of the curves becomes reversed, and the highest one at 2700 Å. is the lowest at 2320 Å. It may be observed in Figure 1 that the curves for this series cross near 2500 Å. Table IV shows the value for the specific alpha at 2500 Å. of the samples from viscosity 3.82 to 101 poises to be 2.04 ± 0.05. Thus, analyses for the amounts of heat-bodied oils of this series in mixtures with raw linseed may be made at 2500 Å. using the value 2.04 for the specific alpha of the bodied constituent.

The procedures described above will not give as accurate results as will the use of the maximum at 2320 Å. of the added bodied oil. However, when the absorption curve of the added oxidized or heat-bodied constituent cannot be obtained, these methods are capable of giving a good estimate of the kind and amount of bodied linseed oil in mixtures with raw linseed oil.

In Table V are given the analyses of mixtures of raw and bodied oils ranging from 5 to 50 per cent of the oxidized or heat-bodied component.

The ultraviolet absorption was measured in a solvent consisting of the purified hexane fraction of petroleum ether (65.5–66.5° C.) containing 10 per cent by volume of ethyl alcohol to bring about solution of the oxidized oil. The petroleum ether was purified for optical use (*15*) by stirring repeatedly with fuming sulfuric acid, washing well, and distilling.

TABLE IV. SPECIFIC ABSORPTION AT 2500 Å. OF HEAT-BODIED LINSEED OILS

Sample	Viscosity *Poises*	Specific α at 2500 Å.	Deviation
D	3.82	2.20	+0.16
G	13.8	2.03	−0.01
I	25.5	2.01	−0.03
K	45.3	2.02	−0.02
N	80.0	2.02	−0.02
P	101.	1.96	−0.08
	Av.	2.04	±0.05
	Raw linseed	0.10	

TABLE V. ANALYSES OF MIXTURES OF BLOWN AND BODIED LINSEED OILS WITH RAW LINSEED OIL

Designation	Specific α at 2320 Å.	Bodied Constituent Found %	Bodied Constituent Added %	Difference in Composition %	Error %
Raw plus blown	0.548	4.88	5.00	−0.12	−2.40
	0.666	7.79	7.50	+0.29	+3.86
	0.756	9.87	10.00	−0.13	−1.30
	0.874	12.85	12.50	+0.35	+2.80
	1.19	21.20	20.00	+1.20	+6.00
	1.50	29.20	30.00	−0.80	−2.66
	1.95	40.00	40.00	0.00	0.00
	2.35	50.00	50.00	0.00	0.00
Raw plus bodied-*in-vacuo*	0.600	5.10	5.00	+0.10	+2.00
	0.730	7.65	7.50	+0.15	+2.00
	0.866	10.35	10.00	+0.35	+3.50
	0.950	12.15	12.50	−0.35	−2.80
	1.31	20.00	20.00	0.00	0.00
	1.80	29.90	30.00	−0.10	−0.30
	2.28	39.40	40.00	−0.60	−1.50
	2.71	48.60	50.00	−1.40	−2.80
Raw plus Blown Bodied-*in-vacuo*	0.34	.....	.....		Av. ±2.12
	4.35	.....	.....		
	5.23	.....	.....		

The raw oil used in these mixtures had a viscosity of 0.40 poise, that of the blown was 1.40 poises, and that of the heat-bodied oil was 16.5 poises. Standard curves were obtained for the raw, oxidized, and heat-bodied oils. The portion of the absorption curves between 2550 and 2900 Å. was used to determine qualitatively the presence or absence of oxidized oil in the mixtures. Quantitative measurements of the blown or heat-bodied oil were then made by using the relatively high absorption maxima at 2320 Å.

The curves presented in Figure 1 relate only to linseed oil produced for this study and were bodied *in vacuo* at 307° C. (585° F.) and blown at 104.4° C. (220° F.). They are not intended for use as standard or reference curves in the quantitative analyses for bodied or blown oils produced under other conditions; it seems likely that the absorption curves for oils bodied or blown at other temperatures, for example, may not be the same. This point requires further study.

Summary

The ultraviolet absorption curves for two series of linseed oils, one heat-bodied at 585° F. and the other blown at 220° F., are presented and discussed.

A distinguishing characteristic between these two series is the development, in the blown series, of an absorption band with a maximum between 2600 and 2700 Å. The specific absorption coefficient of this band increases with the viscosity, on blowing with air, until a viscosity of about 4.5 poises is attained. After this stage of the process, continued blowing causes practically no change in the specific absorption coefficient at 2700 Å., although the viscosity continues to increase. Another absorption band with a maximum at 2320 Å. develops in the blown series. The specific absorption coefficient of this band continues to increase with the viscosity.

The development in the heat-bodied series of a strong absorption band with a maximum at 2320 Å., attributed to the formation of conjugated octadecadienates, takes place with relatively little change in the viscosity. On further heating the specific absorption coefficient of this band decreases gradually as the viscosity increases.

The presence of an absorption band at 2600 to 2700 Å. may be used to distinguish between linseed oil polymerized *in vacuo* and oxidized linseed oil in mixtures with raw linseed oil.

By use of the specific absorption coefficient at 2320 Å., quantitative analyses of oxidized or heat-bodied linseed oil, ranging from 5 to 50 per cent, in mixtures with raw linseed oil have been made with an average percentage error of ±2.12.

The physical constants for each of the samples are given. The acid number, specific gravity, and refractive index increase with the viscosity in each case, while the iodine number drops.

Acknowledgment

The series of heat-bodied and blown linseed oils, together with their physical constants, were made available through S. O. Sorenson of the Archer-Daniels-Midland Co., to whom the authors wish to express their appreciation.

They also want to thank F. P. Zscheile for helpful suggestions made by him during the progress of the work.

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Estimation of Aliphatic C—H Content of Toluene through Its Infrared Absorption

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WHEN toluene is subjected to such reactions as nitration, both the quality of the product and the hazards during the manufacturing operations are dependent upon the purity of the toluene used as the raw material.

The usual physical tests for purity—boiling point, distillation range, refractive index, and density—are not particularly sensitive to small amounts of impurities, and the chemical tests, such as the determination of the bromine number and the sulfonation test, while useful and specific for the impurities encountered, are time-consuming. These tests are conducted in order to estimate the quantities of paraffins, naphthenes, and olefins present in the samples of toluene. These impurities are the substances which exert the greatest influence upon the quality of the nitration products and upon the manufacturing operations. Any method for their estimation which was more sensitive or less time-consuming than the usual tests would be of value.

The use of infrared absorption spectra is not offered as a complete single test for all the impurities found in toluene but only to show that the method has possibilities. The speed, ease, and sensitivity of spectroscopic methods are well known.

First, let us consider the aliphatic nature of the paraffins, naphthenes, and olefins, which are related, in that all the compounds in each class have aliphatic carbon-hydrogen groups present in their structures. Several investigators (*1*, *3*, *4*, *6*) have observed that aromatic C—H vibrations occur in the 3.2μ region of the infrared spectrum while aliphatic C—H vibrations occur in the 3.4μ region. It has also been found that the extinction coefficient per C—H bond is greater in the case of the aliphatic vibration. These differences in the behavior

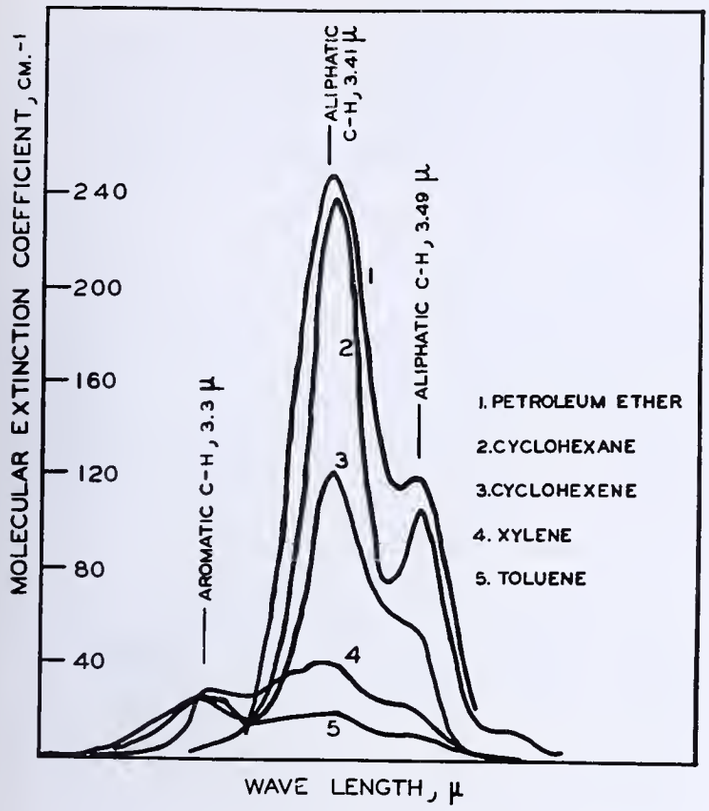

FIGURE 1. ABSORPTION SPECTRA

of the aromatic and aliphatic C—H vibrations should make it possible to detect rather small quantities of aliphatic material in the presence of aromatic substances. Inasmuch as toluene itself has an aliphatic CH_3 group, it is necessary to measure the absorption of the aliphatic C—H over and above that already present in the toluene molecule. This offers no great difficulty, as solutions of toluene can be used as standard absorbents and the presence of aliphatic C—H other than that present in pure toluene will then increase the absorption in the aliphatic region above that normally present in toluene.

Aliphatic C—H may be present in compounds of sulfur such as the sulfides and mercaptans, which may occur in quantities equivalent to 0.1 per cent of sulfur, depending upon the source of the toluene.

The quantity of —SH-bearing compounds may be estimated by determining the absorption in the 3.85μ region where the —SH frequency appears. The C=S frequency occurs between 6.5 and 6.8μ. Compounds containing this group may be studied in the 6.5 to 6.8μ region or possibly in the region of the second harmonic which would occur between 3.25 and 3.4μ. A similar estimation of —OH-bearing compounds may readily be obtained by studying the absorption in the 2.75μ region, while C=C may be estimated by studying the 6.1μ region.

Inasmuch as all these compounds have a number of aliphatic CH groups as well as the various other groups, a relatively simple test, such as the determination of the aliphatic C—H content, should give an indication of the purity of the toluene sample.

Experimental

The authors have compared the infrared absorption spectra of various samples of toluene with toluene purified by the method of Schwalbe (*5*) which is taken as a standard. Samples have been obtained from the stocks possessed by this laboratory and from the Atlas Research Laboratory of the Atlas Powder Company, which kindly permitted the authors to use the results of their analyses of two samples.

The authors have also studied the effect upon the 3.1 to 3.5μ infrared absorption spectrum of toluene of the presence of several molecules, including cyclohexane, cyclohexene, petroleum ether, and *m*-xylene. The first two substances were chosen because the authors were informed that naphthenes and unsaturates cause considerable trouble during the nitration of toluene and these materials were available in a reasonable degree of purity. The petroleum ether was used as a source of aliphatic C—H and because of its boiling range its constituents could be present in some samples of toluene. *m*-Xylene might possibly be a contaminant of toluene and it represents a type of impurity.

The toluene, which was purified by the method of Schwalbe (*5*), had a boiling point of 110.5° C. The cyclohexane was from the Eastman Kodak Company and after several fractionations had a freezing range from 6.3° to 6.5° C. The cyclohexene was obtained from the organic division of this laboratory and boiled between 82.1° and 83.3° C. The petroleum ether was the commercial fraction boiling at 90–110° C. The *m*-xylene was the commercial product which boiled at 138–139° C. after several fractionations.

The absorption spectra were obtained by the technique described previously (*2*, *3*). Carbon tetrachloride solutions of the materials were used and the absorption spectra were obtained by means of the Illinois rock-salt prism spectrometer.

Results

In Figure 1 are shown the absorption spectra of pure toluene, cyclohexane, cyclohexene, petroleum ether, and *m*-xylene. The petroleum ether was assumed to be a mixture of equal quantities of C_7H_{16} and C_8H_{18}. The intense aliphatic C—H absorption of petroleum ether, cyclohexane, and cyclohexene is clearly shown by these curves. It is this high molecular extinction coefficient of the aliphatic 3.4μ C—H, as compared with the relatively weak aromatic C—H in the 3.3μ region, that makes possible the detection and estimation of the quantity of C—H-bearing material in the samples of toluene.

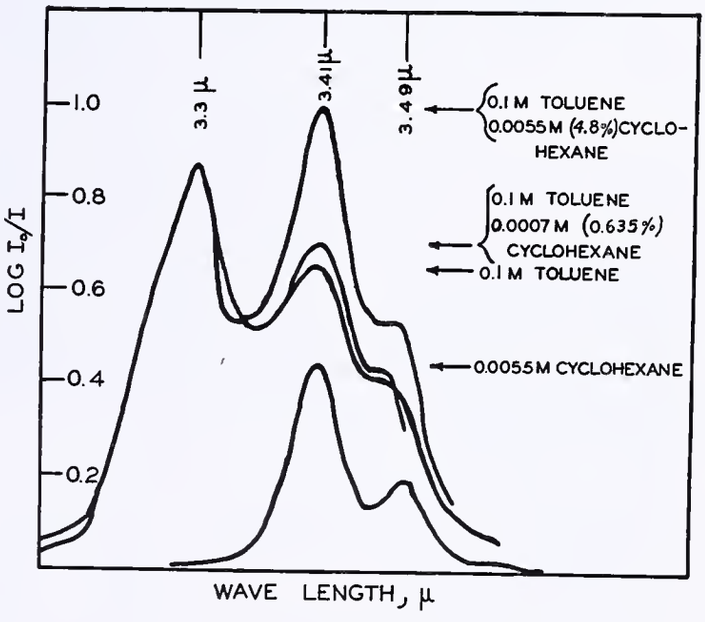

FIGURE 2. ABSORPTION SPECTRA

In Figure 2 curves are shown for different concentrations of cyclohexane added to a 0.1 molar solution of pure toluene. The relative heights of the peaks in the 3.4μ region indicate that the effect of the addition of various concentrations of cyclohexane is what might be expected if Beer's law were applicable.

To test the applicability of Beer's law cyclohexane was added to 0.1 molar toluene solutions in quantities sufficient to produce solutions 0.00012, 0.0006, 0.0012, and 0.0024 molar with respect to the cyclohexane. The logarithms of the ratios of the intensi-

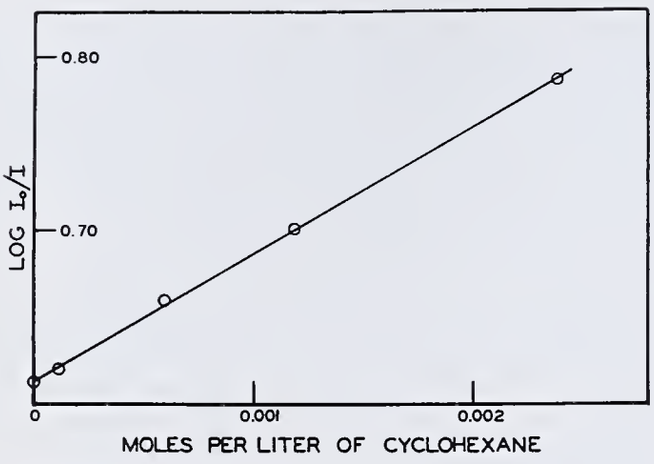

FIGURE 3

ties at the 3.4μ absorption peak of these solutions were determined, using a constant cell thickness. For strict adherence to the customary meanings of the symbols in Beer's law as written, $k = \frac{1}{c}\left(\log \frac{I_0}{I}\right)$, it would be necessary to obtain values for the intensity of radiation through a cell containing carbon tetrachloride plus toluene plus impurity (cyclohexane in this case). This intensity can be represented by I_{a+b+c} using a, b, and c for carbon tetrachloride, toluene, and the impurity, respectively. It is also necessary to measure the intensity of radiation through a cell containing carbon tetrachloride and toluene, which can be represented by I_{a+b}. With these two values of the intensity of radiation it is possible to formulate a statement of Beer's law in the usual form.

For convenience, however, the authors have determined the intensity of radiation through a carbon tetrachloride solution of toluene plus the impurity and through a cell containing only carbon tetrachloride. These values may be represented by I_{a+b+c} and I_a, respectively.

Now

$$\frac{I_a}{I_{a+b+c}} = \frac{I_a}{I_{a+b}} \times \frac{I_{a+b}}{I_{a+b+c}}$$

but $\frac{I_a}{I_{a+b}}$ is constant; therefore a plot of the log of $\frac{I_a}{I_{a+b+c}}$ *vs.* the concentration of the impurity will be proportional to a plot of the variables usually encountered in Beer's law, $\frac{I_{a+b}}{I_{a+b+c}} = \frac{I_0}{I}$. If these values fall upon a straight line it will be evidence of the validity of the law. Such a plot is shown in Figure 3, where the experimental points fall sufficiently close to the line to enable the strict application of Beer's law. This adherence to the law is reasonable, inasmuch as only slight association is to be expected in carbon tetrachloride solutions of the substances examined.

When commercial samples of toluene are to be examined through their infrared absorption, the hydrocarbon impurities are of unknown character. The success of the nitration of toluene depends, however, upon the presence in sufficiently low quantities of olefins, paraffins, and naphthenes. These substances have a strong infrared absorption at 3.4μ due to aliphatic C—H vibrations, and a comparison of the height of this absorption peak with the height of the peak exhibited by pure toluene may be used as an additional aid in the estimation of the suitability of toluene for nitration. The absorption exhibited by the unknown hydrocarbons can be calculated as equivalent to the absorption exhibited by known quantities of a definite compound. The authors have used the absorption shown by cyclohexane as their standard, both because it is a naphthene and naphthenes cause trouble in nitration and because the cyclohexane was of high purity.

Toluene samples A and B and their analyses were furnished through R. Max Goepp, Jr., and Sol Soltzberg of the Atlas Research Laboratory of the Atlas Powder Company. These samples had been subjected to a chemical analysis including the following determinations: olefin content by bromine number, paraffins and naphthenes by sulfonation, and Podbielniak distillation with density and refractive index determinations on each cut.

The composition of these two samples is as follows:

	A	B
Toluene (by difference), %	98.58	99.70
Olefin, %	0.06	0.02
Paraffins and naphthenes, %	1.36	0.28

The results of the analysis justify the further conclusions that there are no xylenes or higher homologs of toluene present in either sample, that the olefin content is practically negligible, but the B sample is more sensitive to 91 per cent sulfuric acid. The color-forming substance in sample B is completely removable by the acid. Furthermore, the residual oils from the sulfonation of the samples of toluene A and B had boiling ranges at 745.9 mm. of 121–122° C. and 117–117.5° C., respectively, and refractive indices of $n_D^{25} = 1.4150$ and 1.4172. The amount of these oils was too small for further identification, but a search of the literature on the boiling points and refractive indices of paraffins and naphthenes having constants similar to those found leads to the conclusion that these residual oils appear to be mixtures of C_8 and C_9 paraffins and naphthenes. Sample A has been nitrated successfully on a commercial scale and sample B has been adjudged suitable for commercial nitration.

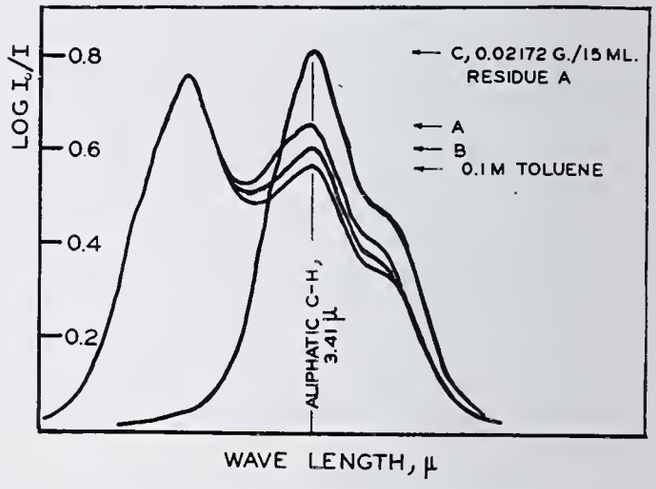

FIGURE 4. ABSORPTION SPECTRA

The absorption spectra of samples A and B and the residue from sample A are shown in curves A, B, and C, respectively, in Figure 4. These spectra were obtained after the spectrometer, a research instrument, had been disassembled and reassembled for an overhauling. The differences in the values of $\log \frac{I_0}{I}$ for pure toluene and those shown in Figure 2 are due to inescapable changes in the resolution of the instrument which develop during this major operation. To estimate the quantity of aliphatics present in the samples after such instrumental changes necessitates the redetermination of a calibration curve. A new curve is not shown, as it would be similar to that in Figure 3.

However, the absorption spectra show that curve A represents a sample of toluene containing aliphatic C—H equivalent to 0.00147 molar (1.44 per cent) cyclohexane and curve B represents a sample containing aliphatic C—H equivalent to 0.0007 molar (0.62 per cent) cyclohexane. These values are of the same order of magnitude as the results of the chemi-

cal analysis. Sample A has been successfully nitrated on a commercial scale, yet it is possible to detect spectroscopically impurities present in even smaller amounts than in sample A, as shown by the spectrum of B. The residue from sample A, curve C, appears to contain practically no aromatic C—H. No attempt has been made to differentiate olefins and naphthenes. The C=C bonds in the olefins could probably be detected by similar procedures in the 10μ region or possibly at 5μ in the second harmonic.

The spectroscopic examination has the usual spectroscopic advantages over the chemical examination for aliphatic C—H such as speed and ease of determination. An examination can readily be completed in 30 minutes. In the concentration region represented by Figure 3 the error introduced by variation in the spectrometer readings could be in the neighborhood of 1 or 2 per cent.

Acknowledgment

The authors wish to express their thanks to A. M. Buswell and W. H. Rodebush of the University of Illinois and R. Max Goepp, Jr., and Sol Soltzberg of the Atlas Laboratory for their interest and help, and to the Atlas Powder Company for permission to use the results of their analysis.

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Determination of Antimony in Lead-Antimony Alloys

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A new bromate method for the determination of antimony in lead-antimony alloys is described and compared with older methods. The method is rapid, convenient, and especially recommended for use on lead alloys that do not dissolve readily in sulfuric acid. The effect of the presence of copper on the antimony determination has been investigated. Under certain conditions copper can act as an oxidation catalyst and thus cause the results of an oxidimetric determination of antimony to be too low; under different circumstances copper can cause high results.

THE method most commonly used for the rapid determination of antimony in lead-antimony alloys is that of Low (*4*) in which the sample is dissolved in concentrated sulfuric acid and potassium bisulfate and, after addition of hydrochloric acid, the antimony is titrated at 10° C. with permanganate ion in the presence of the lead chloride-sulfate precipitate. This method has been applied to the analysis of 1 per cent antimony alloys and difficulty in obtaining complete sulfation of certain alloys is reported (*9*). In agreement with this finding the authors have also encountered alloys which do not dissolve completely, even though a preheated solution of sulfuric acid and potassium bisulfate is employed (*9*). Further work has revealed that alloys containing appreciable quantities of copper—e. g., 0.05 per cent—are always more difficult to sulfate than copper-free alloys; and that the metallic residue obtained in such cases is relatively rich in antimony and copper, suggesting the presence of an antimony-copper compound, probably Cu_2Sb.

The problem of obtaining complete solution of the sample has led analysts to increase the time and temperature of heating during the sulfation step, or to add an oxidizing agent such as cupric ion. In subjecting the sample to such treatments the assumption has been made that the subsequent titration of the trivalent antimony is unaffected. In the experimental section of this paper data are presented which show that prolonged heating at approximately 320° C. during sulfation results in low values for antimony in lead-antimony alloys containing copper; and that the low results (Tables V and VI) are due to oxidation of part of the trivalent antimony, catalyzed by copper ions (*8*, *11*). Data are also presented which show that the use of cupric ion as an oxidizing agent in the solution of the sample will lead to a positive error unless the cuprous ion formed is reoxidized before titration.

In view of the possibilities of error in the Low method resulting from incomplete sulfation of the sample and from partial oxidation of the antimony to the pentavalent form, it seemed desirable to develop a new, rapid method in which these errors would be closely controlled. The time element eliminated the possibility of using a method involving solution of the sample in hydrochloric acid and bromine, followed by reduction of the antimony and titration with standard bromate solution (*1*, *2*, *7*). While this method is long, it still has several advantages over the Low permanganate method, chief of which is that the alloy is taken completely into solution before titration. It occurred to the authors that it might be possible to combine the best features of the two methods, and this has proved practicable. The method developed involves the following simple operations:

Solution of the alloy in sulfuric acid and potassium bisulfate.
Addition of sufficient potassium chloride and hydrochloric acid to effect complete solution of all metal and metal salts by conversion to chlorides.
Titration of the antimony at 80° to 90° C. with bromate ion.

The method (henceforth called the bromate method) eliminates errors resulting from incomplete sulfation and occlusion of antimony, by taking the sample completely into solution. [Somewhat similar methods have been described by Pugh (*5*), Robinson (*6*), Shreider (*10*), and Vasil'ev and Stutzer (*11*).] The use of air-saturated distilled water in the dilution step was found to provide sufficient oxygen to effect

Table I. Comparison of Bromate and Permanganate Methods

Alloy No.	Antimony		Copper
	Bromate	Permanganate	
	%	%	%
1	1.00	1.00	0.5
2	0.78	0.78	0.05
3	1.02	1.03	0.05
4	0.92	0.92	0.05
5	1.00	0.99	< 0.002
6	1.02	1.02	< 0.002
7	1.00	1.00	< 0.002
8	0.91	0.91	< 0.002
9	0.98	0.98	< 0.002

solution of all metal and to reoxidize any cuprous ion formed. The possibility of oxidation of trivalent antimony during sulfation is recognized and the error minimized by limiting the time of heating during sulfation to 7 minutes, a period found by experiment to be sufficient to expel the sulfur dioxide formed in the solution of the sample. The method is precise, convenient, and considerably more rapid than any of the modifications of the Low permanganate method known to the authors. Duplicate determinations may be completed in 20 minutes as compared to 45 to 60 minutes by the modified Low permanganate method.

Table II. Control Method Applied to Synthetic Samples of Known Composition

Antimony Present	Antimony Found	Error
Mg.	*Mg.*	*Mg.*
5.0	5.0	0.0
3.9	4.0	+0.1
3.0	3.1	+0.1
8.5	8.5	0.0
5.6	5.7	+0.1
3.4	3.4	0.0

Bromate Method

Reagents. *Methyl Orange Solution.* Dissolve 0.1 gram of methyl orange in 100 ml. of distilled water.

Potassium Bromate Solution (0.05 N). Dry a sample of finely ground, pure, bromide-free potassium bromate at 180° C. for an hour (*3, 12*). Dissolve 2.7837 grams of the dried salt in distilled water and dilute to 2 liters in a volumetric flask.

As further check, the solution may be standardized against pure arsenious oxide (Bureau of Standards Sample 83).

Procedure (suitable as written for the determination of antimony in 1 per cent antimony–lead alloys). Weigh out 2 grams of the milled alloy and transfer to a 500-ml. Erlenmeyer flask. Add 10 ml. of sulfuric acid and 5 grams of fused potassium bisulfate, place on a hot plate, and heat for 7 minutes. (The temperature of the solution at the end of this heating period must be 320° ± 5° C.) Remove flask from hot plate and cool.

Add 230 ml. of cold distilled water (air-saturated), washing down the sides of the flask in the process. Add 15 grams of potassium chloride and 20 ml. of hydrochloric acid, heat just to boiling to dissolve all metal and metal salts, and boil vigorously for 1 minute, shaking (by rotation) the contents of the flask once or twice during the boiling. [If for any reason a black metallic residue is obtained which does not dissolve readily in the shaken, boiled chloride mixture, proceed as follows: To the hot solution add 2 ml. of copper sulfate solution (50 grams of copper sulfate pentahydrate per liter of water). Boil and shake until all the metal dissolves. Dilute to 350-ml. volume with hot water, heat to 90° C., bubble oxygen through the solution for 5 minutes, and titrate at 85° to 90° C.]

Add 100 ml. of air-saturated distilled water. Titrate slowly at 85° to 90° C. with standard bromate solution (0.05 *N*) using not more than 3 drops of methyl orange indicator.

Ml. of bromate solution × 0.1522 = per cent of antimony in sample.

Experimental

In order to compare the new bromate method with the permanganate method the authors have analyzed several representative 1 per cent antimony–lead alloys by the two methods. The permanganate method used was that of Shaw, Whittemore, and Westby (*9*), except that the heating period during sulfation of the sample was reduced from 30 to 7 minutes to minimize oxidation of trivalent antimony.

Table I gives a comparison of the antimony contents of nine alloys, obtained with the two methods. The alloys contain copper together with traces of other metallic impurities such as silver, bismuth, nickel, and iron. Arsenic and other interfering elements were not present in significant quantities. Owing to the high copper content of alloy 1, oxygen was passed through the solution in this case for 5 minutes immediately before titration. The data show that the two methods give almost identical results when approximately the same sulfation conditions are used.

Neither the new bromate nor the modified permanganate method can be tested on synthetic solutions. It was therefore necessary to develop a control method of known precision and to calibrate the two rapid methods against this method by the analysis of a common sample.

Control Method (suitable as written for the analysis of antimony in 1 per cent antimony–lead alloys).

Weigh out 300 mg. of the milled alloy and transfer to a 500-ml. Erlenmeyer flask, add 15 ml. of sulfuric acid, heat to boiling and boil gently until the sample is completely decomposed. Heat vigorously on a flame to dissolve all traces of metal and lead sulfate. Add 25 mg. of hydrazine sulfate to the solution and heat vigorously on a flame until the solution is reduced to a volume of approximately 10 ml. Cool.

Add 220 ml. of distilled water, 20 ml. of hydrochloric acid, and a few glass beads, heat to boiling, and boil vigorously for 1 minute, shaking (by rotation) the contents of the flask once or twice during the boiling. Titrate the antimony with potassium bromate solution (0.01 *N*) using methyl orange as indicator. Determine blank on the reagents.

[Ml. of $KBrO_3$ − blank] × 0.2028 = per cent of antimony in sample

Table III. Reproducibility of Permanganate, Bromate, and Control Methods

(Applied to alloy 9)

	Permanganate Method		Bromate Method		Control Method	
	Antimony found	Deviation	Antimony found	Deviation	Antimony found	Deviation
	%	%	%	%	%	%
	0.98	0.00	0.97	−0.01		
	0.97	−0.01	0.99	+0.01	0.972	−0.003
	0.97	−0.01	0.97	−0.01	0.970	−0.005
	0.97	−0.01	0.98	0.00	0.973	−0.002
	0.98	0.00	0.97	−0.01	0.985	+0.010
	0.99	+0.01	0.97	−0.01	...	.....
	0.96	−0.02	0.97	−0.01	...	.....
	0.98	0.00	0.98	−0.00	...	.....
Mean	0.98	±0.007	0.98	±0.008	0.975	±0.005

Table IV. Effect of Temperature and Time of Heating during Sulfation

(Upon apparent antimony content of alloy 9 by the bromate method)

Antimony Found		Antimony Found	
Heating period, 10 minutes	Temperature	Heating period, 7 minutes	Temperature
%	° C.	%	° C.
0.97	325	0.98	325
0.96	325	0.98	325
0.97	325	0.97	325
0.97	325	1.00	300
0.97	325	0.99	300
0.97	325	0.99	290
0.98	325	1.02	290
		1.02	290

The data in Table II were obtained by the control method on synthetic mixtures of known composition closely approximating the composition of the lead-antimony alloys to be analyzed.

Table III shows a comparison of the reproducibility of the bromate, permangante, and control methods on alloy 9. The agreement is satisfactory.

In Table IV data on the effect of temperature and time of heating during sulfation upon the results obtained by the bromate method on alloy 9 are shown. At temperatures below 325° C. there is evidence of incomplete removal of sulfur dioxide, leading to high results. There is a slight trend to lower results when the time of heating is increased from 7 to 10 minutes.

Table V shows the effect of copper as a catalyst in the oxidation of antimony during sulfation of the sample in the permanganate method. The heating periods were timed from the moment the sample was dropped into the hot acid solution.

Table V. Effect of Copper and Time of Heating
(Permanganate method)

Alloy No.	Cu	Antimony Found: Heating period, 7 minutes	Antimony Found: Heating period, 45 minutes	Antimony Lost
	%	%	%	%
1	0.5	1.00	0.91	0.09
2	0.05	0.78	0.72[a]	0.06
3	0.05	1.03	0.95	0.08
4	0.05	0.92	0.85	0.07
5	< 0.002	0.99	0.97	0.02
6	< 0.002	1.02	0.99	0.03
7	< 0.002	1.00	0.97[a]	0.03
8	< 0.002	0.91	0.89	0.02

[a] After titration these samples were treated with sulfur dioxide, boiled vigorously for 15 minutes,, and retitrated at 10° C. Values found were: 2 = 0.79% Sb, 7 = 1.00% Sb.

Table VI. Effect of Presence of Copper on Oxidation of Antimony in Sulfuric Acid Solution

H_2SO_4 Present	Time of Heating	Copper Present	Antimony Present	Antimony Found	Error
Ml.	*Min.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
15	10	10	5.0	3.9	−1.1
25	30	10	5.0	3.5	−1.5
25	30	10	5.0	4.8[a]	−0.2
25	30	10	5.0	4.7[a]	−0.3
15	10	1	5.0	4.7	−0.3
25	30	1	5.0	4.3	−0.7
15	10	0	5.0	5.0	±0.0
25	30	0	5.0	5.0	±0.0

[a] After heating for 30 minutes the samples were treated with 100 mg. of hydrazine sulfate, heated on a flame to expel approximately 5 ml. of sulfuric acid, cooled, diluted, and titrated as usual.

In order to investigate further the effect of copper ions on the oxidation of antimony in sulfate solution the following experiment was carried out.

Five milligrams of antimony were dissolved in a solution of 15 or 25 ml. of sulfuric acid and 5 grams of potassium bisulfate in a 500-ml. Erlenmeyer flask. Weighed amounts of copper (as copper sulfate) were added. The samples were heated at 320° C. for 10 to 30 minutes, then cooled, diluted to 300-ml. volume with water, treated with 20 ml. of hydrochloric acid, and titrated at 90° C. with potassium bromate solution (0.01 *N*), using methyl orange as an indicator.

The results, corrected for a titration blank, are shown in Table VI.

To determine whether trivalent antimony in hot chloride solution is readily subject to air oxidation, a series of low-copper and high-copper alloys was analyzed by the bromate method as recorded above, except that oxygen was bubbled through the solution for 5 minutes just before titration. The results, contained in Table VII, show no significant difference

Table VII. Effect of Oxygen on Trivalent Antimony in Hot Hydrochloric Acid Solution

Alloy No.	Copper	Antimony: With oxygen	Antimony: Without oxygen
	%	%	%
2	0.05	0.78	0.78
3	0.05	1.01	1.02
4	0.05	0.92	0.92
5	< 0.002	1.00	1.00
6	< 0.002	1.02	1.02
8	< 0.002	0.91	0.91

Table VIII. Effect of Cuprous Ion in Bromate Method

Antimony Found: With oxygen	Antimony Found: Without oxygen
%	%
0.93	1.12
0.92	1.20
0.92	0.95
0.92	1.01

in comparison with those obtained by the method as normally used—i. e., without the use of oxygen. This is contrary to the finding of Zintl and Wattenberg (*12*).

To show that an error can result from the use of cupric ion in the solution of a sample, alloy 4 was analyzed as recorded in the bromate method, except that after dilution of the sample with water and chloride, 2 ml. of copper sulfate solution (50 grams of copper sulfate pentahydrate per liter) were added and the solution was boiled and shaken until all metal dissolved. The sample was then diluted to 350-ml. volume with hot water and (1) saturated with oxygen for 5 minutes before titration; or (2) titrated without the use of oxygen. The results, contained in Table VIII, show that precautions must be taken to reoxidize any cuprous ion formed during solution of the sample.

Summary

A new bromate method for the determination of antimony in lead-antimony alloys of low antimony content has been developed. For most alloys it is no more precise than the permanganate method (*9*), but it is considerably more rapid and convenient. The new method is especially suitable for the analysis of alloys that do not dissolve readily during sulfation.

The effect of copper in the antimony determination has been investigated. Under certain conditions copper can act as an oxidation catalyst and thus cause the results of an oxidimetric determination of antimony to be too low; under different circumstances copper can cause high results. Precautions which will eliminate or minimize these errors are specified.

With slight modifications in procedure the new bromate method can be used for the determination of antimony in lead alloys of high antimony content and in tin and lead-tin alloys.

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Photometry in Spectrochemical Analysis

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SINCE the photographic plate is the most widely used medium for the measurement of radiant energy, the relation between intensity and the photographic effect produced has been very extensively studied (*4*). Despite this work, however, the spectrochemical analyst frequently must reinvestigate the procedures, in order to establish their applicability for his work. In this paper are described the investigations made by the authors and the conclusions reached.

Definitions

The following definitions and symbols are used in this discussion:

i is the intensity of radiant energy which strikes the photographic plate. Strictly speaking, the plate does not measure intensity (energy per unit time) but rather integrated intensity or total energy (*10*). In most analytical work, however, an internal standard is employed to permit correction for variations in the exposure conditions, and all energies are stated in terms of the relative intensities of analysis and internal standard lines. Thus it makes no difference whether the effect is given in terms of energy per unit time or total energy. The absolute value of i is never determined in analytical work. All intensity values are stated in relative terms based upon the empirical scale given by a plate calibration curve.

I and I_o refer to the intensity of light transmitted by a developed photographic plate, as measured by a microphotometer. I refers to the photometer reading for the light transmitted by an exposed portion of the plate, usually a spectrum line, and I_o to the photometer reading for a clear unexposed portion of the same plate. In all measurements the ratio of the two readings is needed to express the photographic effect. The ratio is expressed in various ways, as transmission, opacity, or density.

Transmission, T, is defined by the expression

$$T = \frac{I}{I_o}$$

Opacity, O, is the reciprocal of transmission.

Density, D, is defined by the relation

$$D = \log_{10} \frac{I_o}{I} = \log_{10} \text{opacity}$$

Density is a more fundamental concept for expressing photographic response than opacity, because density is a linear function of visual response. Furthermore, density is proportional to the number of developed silver grains in the emulsion (*5*).

The relation between density and light intensity is usually expressed by an H. & D. curve (*5*), which is a plot of density *vs.* log relative intensity or relative exposure. Examples of such curves are shown in Figures 2 and 5. The basis for this method of plotting plate response lies in the fact that the curve obtained has a long linear portion which for some plates may extend from a density of above 0.5 to above 2.0. Furthermore, this type of plot is convenient when one wishes to obtain the intensity ratio of two lines, since the difference along the log exposure axis gives directly the logarithm of the intensity ratio of the two lines. Another useful type of plate response curve is shown in Figure 1, where density is plotted *vs.* intensity (or exposure). The advantage of this method is that any density value can be converted directly into the corresponding intensity value. A disadvantage is, of course, that the curve has no linear portion.

Plate Calibration

Photographic intensity measurements are made by comparison of the density of the line to be measured with the density produced by a line of known relative intensity. Since the effect produced by a given number of light quanta depends upon the properties of the plate, the wave length of the light, the developer used, the temperature and time of development, and many other factors, it is necessary to have for every plate a calibration curve or H. & D. curve. Such a curve is constructed by placing on the plate a series of exposures of known relative intensity, measuring the densities of the resulting lines, and plotting the densities as a function of the intensity.

The comparison of intensities by means of the photographic effects produced is subject to several kinds of errors (*4*). Strictly speaking, such a comparison is accurate only when the exposures are made simultaneously and nonintermittently; the wave lengths of the compared radiations are equal; the comparison is made between lines of equal density; and the comparison is made between lines lying on adjacent portions of the emulsion.

It is not possible to fulfill these conditions in routine analytical work. The plate calibration exposures are often intermittent and cannot be made simultaneously with the analysis exposures. Lines to be compared with one another are often widely separated on the plate. Usually it is necessary to make comparisons between lines of widely different densities. It is necessary, therefore, to determine to what extent the departure from ideal conditions will affect the accuracy of intensity measurements.

The first point to be investigated is the method for making exposures of known relative intensity for the construction of the calibration curve. There are three general methods for doing this.

STEPPED SECTOR. A rotating sector with stepped openings of known relative values is placed before the slit, which is uniformly illuminated from top to bottom. The spectrum obtained has steps of known relative exposure times. A source giving either continuous light or lines may be used for illumination.

STEPPED WEAKENER. A neutral weakener, made by sputtering a quartz plate with some reflecting or absorbing material in steps of known relative transmission, is placed before the slit. Either continuous or monochromatic radiation may be used.

STEPPED SLIT. The spectrograph slit is opened wide and is replaced by a slit with a series of stepped openings of known widths. A source of continuous radiation must be used for illumination, in order that the number of quanta striking unit area of the plate may be proportional to the width of the slit in each step.

Each of these methods has advantages and disadvantages. The stepped sector is easiest to construct and use, and it gives the same relative transmission in the various steps regardless of the wave length. The sector has long been mistrusted, however, because it gives intermittent exposures and because of possible reciprocity failures due to the unequal time of illumination for the various steps. The stepped weakener is theoretically the soundest method, but it is a research job to prepare and calibrate the absorbing layers and to ensure that the relative transmissions of the steps are the same for all wave lengths (*1*). The stepped slit is difficult to make accurately, since the smallest opening must be not over 0.125 mm. in width if one is to obtain five steps which have a transmission ratio of two. A further disadvantage is the difficulty of obtaining a suitable source of continuous radiation in the ultraviolet region commonly employed for spectrochemical analyses. The source must have a fairly uniform intensity over the whole wave-length region of interest, since the spectral purity of the image is a function of the slit width in the various steps; in a region of abrupt change of intensity the image given by the widest step will be affected to a greater extent than the image from the narrow steps.

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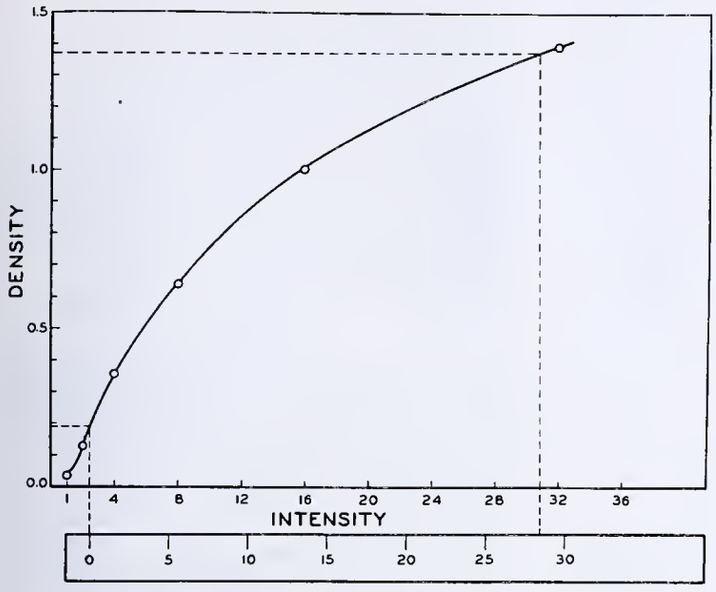

FIGURE 1. DENSITY-INTENSITY PLOT AS USED FOR BACKGROUND CORRECTION

Recently the step-sector method has grown in favor, since Webb (*14*) has shown that reciprocity failures disappear when illumination is made by intermittent exposures of high interruption frequency. His results may be summarized in the statement (*4*): "If the frequency of interruption is sufficiently high, an intermittent time-scale exposure becomes identical with an intensity-scale exposure. The critical frequency is a rate of interruption sufficiently high that each individual silver halide grain receives on the average approximately one quantum per flash." Experiments were performed to test whether, under the conditions employed in analysis, this equivalence between intermittent and uninterrupted light exists. In these experiments a comparison was made between plate calibrations obtained by use of the stepped slit and by the stepped sector.

A five-step slit was constructed to fit in the wedge guides of the spectrograph. Each step was 2 mm. in height; the widths of the steps were made approximately 0.125, 0.25, 0.50, 1.0, and 2.0 mm. The exact widths were determined by measurement with a filar micrometer at various positions and an average value was taken for each step. Illumination was obtained from an underwater spark between tungsten electrodes (*11*) with a Tesla coil discharge. This source gave continuous radiation extending down to a wave length near 2000 Å., with a fairly uniform distribution of intensity over the wave-length range which was used in this study, 2500 to 3400 Å. The source was placed about 2 meters from the slit of the Littrow spectrograph used with no intervening lens, so that uniform slit illumination was obtained from top to bottom. Exposure times of 5 to 8 minutes gave spectra of suitable density for measurement. Process plates were used, developed for 2 minutes at 18° C. with Eastman D9 developer. The densities of the various steps were measured at a wave length of about 3000 A. and H. & D. curves were constructed by plotting densities *vs.* $\log_2$ relative exposures. This log scale was chosen because the slit was made on this basis, the log values for the transmissions of the various steps being 0, 1, 2, 3, and 4.

A seven-step rotating sector was constructed from a brass disk. The stepped openings gave relative transmissions of 1, 2, 4, 8, 16, 32, and 73 (the last due to an error in cutting). Each step was 1.5 mm. in height. The sector was mounted on the shaft of a small motor (operated at 1800 r. p. m.) and the whole assembly was so placed on the optical bench that the sector stood at a distance of 1 cm. from the slit. Separate exposures were made with the continuous source described above and with a mercury vapor lamp operated on direct current. H. & D. plots of density *vs.* $\log_2$ relative exposure were made as in the stepped-slit experiments, at a wave length of about 3000 Å. for the continuous source and for the mercury 2967 A. line for the spectrum made from the arc.

The three H. & D. curves obtained, respectively, for the stepped slit, the stepped sector with continuous radiation, and the stepped sector with monochromatic radiation, were found to be within experimental error exactly equivalent; all three had the same slope in the linear portion and were very nearly parallel to one another in the toe portion. In further experiments, using the mercury vapor lamp as source, interruption frequencies as low as 100 per minute were found to give curves identical with those obtained at high frequencies. This result is in agreement with Malpica (*7*), who reports that he finds no difference in plate gamma for sector speeds varying from 1 to 3600 interruptions per minute.

From the results of these experiments, it appears that the stepped sector may be trusted as a calibration source; there seem to be no errors of reciprocity failure introduced by its use in the wave-length region studied, provided the total light intensity is low. Therefore, since it is easy to construct and use the sector, this method is recommended for plate calibrations. The following conditions have been found to give good results:

A suitable source of line radiation is mounted at a distance of 1.5 to 3 meters from the slit, so that uniform illumination is assured. No lens is used between the source and the slit. The source is operated at such intensity that an exposure of 3 to 6 minutes gives lines of suitable density for measurement. The sector is mounted on the shaft of a small induction motor and permanently left in position just before the slit; during the calibration exposure it is rotated, but for analytical exposures the sector is left in the open position. The source is mounted on a pivoted arm, so that it can be moved back and forth during the exposure, in order that the light beam may travel completely across the collimator lens. With the source in a fixed position only a narrow area of the lens and prism is utilized and the lines obtained are not flat (a fixed source may be used if a long-focus lens is placed just before the slit, so that the light is diffused to cover the collimator lens).

The source must give well-separated lines in the desired analytical region and must give a spectrum which is entirely free of background adjacent to the lines. A mercury vapor lamp, operated on direct current, was first used in this work, with excellent results. Later, it was found that equally good results were obtained from a 5-ampere arc between brass electrodes. Massive electrodes (diameter 15 to 20 mm.) must be used, so that heating of the anode to incandescence is avoided.

It has been found convenient to mount the calibration source permanently in a position at right angles to the external optical path and to use a right-angle quartz prism for bringing the light into the slit for the calibration exposures. This arrangement permits the operator to leave all equipment on the optical bench in place during the calibration exposure and only the right-angle prism need be removable.

It has been found, from the results with many plates, that calibration of every plate is unnecessary; in general it is sufficient to calibrate one plate from a box or from a lot, provided that a standardized procedure is used for development. Exact control of both the developer temperature and the agitation is important. Good results have been obtained by a rapid rocking procedure and also by brushing the plate during development. In the latter method the tray is left motionless and the emulsion is brushed by long sweeps of a camel-hair brush, so that each area receives approximately the same number of strokes.

It is not necessary to use a seven-step sector, as described above. For most purposes it is sufficient to use a sector with four steps, provided an uninterrupted portion of the beam is allowed to pass above the sector, so as to make a fifth step. The exposure should be so timed that the desired line has a density of 0.04 to 0.06 in the lowest exposure step. The density of the highest exposure step will then fall on the linear portion of the H. & D. curve, which can be extrapolated by

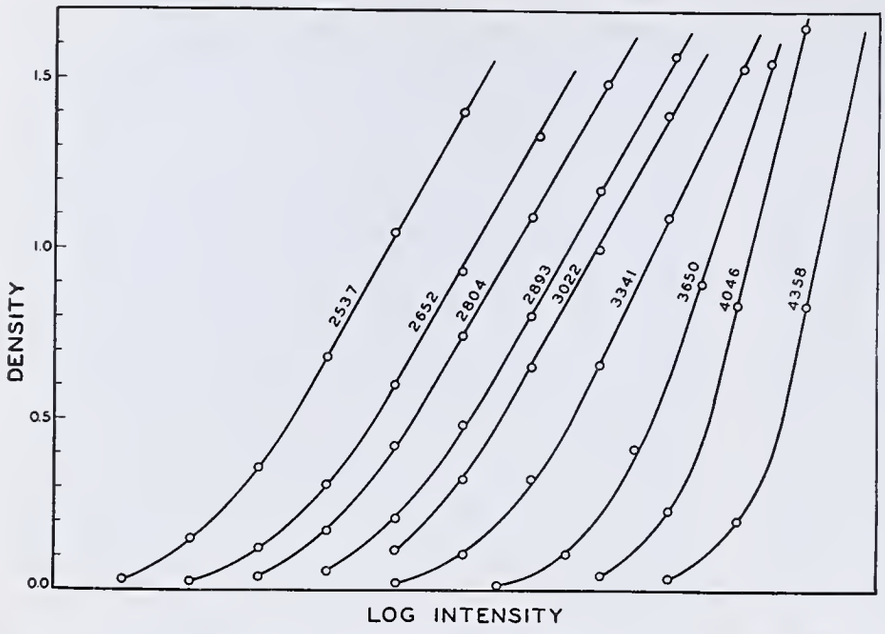

FIGURE 2. VARIATION OF GAMMA WITH WAVE LENGTH FOR PROCESS PLATE

prolonging the linear portion up to a density of 2 or above. The validity of the extrapolation can, at any time, be confirmed by construction of a curve for a stronger line of the same spectrum and plotting these data on the same scale, so as to extend the curve to higher densities.

The observation that the plate gamma (slope of the H. & D. curve) is the same for monochromatic and continuous light does not agree with results obtained by Strock (*12*). In experiments made with the copper 5782 Å. line and with continuous light of the same wave-length region, he found the slope of the density–log exposure curve to be considerably greater for the line than for the continuous radiation. His explanation is that penetration of the developer is different for the spectrum line and for the extended band of continuum, the narrow line receiving more developer than an equal area of the continuum. The reason for the discrepancy between Strock's results and those given above is not clear; a possible explanation is that his work was done on sensitized plates, the present work on unsensitized plates. Doubtless the response of the two types of plates is different, as related to speed of penetration of the developer. Certainly no evidence for the existence of the Eberhard "nachbar" effect was noted in the present experiments; the Process plate gives the same response per quantum of light (at a given wave length in the ultraviolet region) regardless of whether the light be monochromatic or continuous. This observation is important in connection with the question of background corrections, for it has been generally assumed, on the basis of Strock's results, that the response is different for background and line radiation. This belief has retarded the successful application of background corrections in spectrochemical analyses.

Variation of Gamma with Wave Length

It is well recognized that plate gamma is a function of wave length, but unfortunately most of the data published about this effect are for wave lengths above 3500 Å. Consequently it was found necessary to investigate the magnitude of the effect in the analytical region 2500 to 3400 Å. This was done for Eastman Process and for Spectrum Analysis No. 1 plates. Results for Process plates are shown in Figure 2. The H. & D. curves are strictly parallel to one another throughout the region 2500 to 3100 Å., but above 3100 Å. the slope of the curves increases with increasing wave length. Similar results were found for Spectrum Analysis No. 1 plates, except that the gamma value is greater in all regions than for Process plates.

Since the plate gamma is constant for the region 2500 to 3100 Å. a single calibration curve suffices for all lines in this region and a single internal standard line may be used for all analysis lines. At higher wave lengths the plate must be calibrated for the same wave-length region as the analysis line and the internal standard line must lie near the analysis line.

Accuracy of Intensity Measurements

The accuracy of spectrochemical analyses is influenced by two types of errors: unequal vaporization and excitation of the constituents of the sample, and errors in the photographic process itself. In order to investigate the magnitude of the latter errors, independently of any errors in excitation, relative intensity ratios were determined for several steps of line pairs in the same spectrum, photographed by means of the stepped sector used for calibration. Obviously, the actual intensity ratio for a given line pair is the same for all steps and any variations found can be attributed to errors in the photographic process. The following experiment was performed:

The spectrum of a mercury vapor lamp was recorded by means of a seven-step sector, in the manner described for plate calibration. A plate calibration curve was constructed for the 2894 Å. line. By means of this calibration curve relative intensity values were determined for all measurable steps of the 2652, 2654, and 2655 Å. lines; intensity ratios were then computed for corresponding steps (Table I). In further tests intensity ratios were determined for the same line pairs from several spectra, taken on different plates. The data are summarized in Table II.

TABLE I. INTENSITY RATIOS

Step	Intensity 2652/2654	Intensity 2652/2655	Intensity 2654/2655
1	2.14	3.47	1.62
2	2.07	3.35	1.62
3	1.97	3.26	1.66
4	2.10	3.36	1.65
5	2.14		
Av.	2.08	3.36	1.64
Av. % deviation	2.4	1.6	1.05

TABLE II. INTENSITY RATIOS

Intensity 2652/2654	Intensity 2652/2655	Intensity 2654/2655
2.14	3.47	1.62
2.07	3.35	1.62
1.97	3.26	1.66
2.10	3.36	1.65
2.14	3.54	1.65
2.14	3.32	1.57
2.14	3.40	1.61
2.14	3.27	1.57
2.10	3.46	1.60
2.10	3.32	1.61
2.07	3.40	1.61
2.13	3.37	1.61
2.09	3.49	1.59
2.19	3.22	1.59
2.04	3.25	1.62
2.03	3.03	1.49
2.07	3.24	1.63
2.00	3.24	1.62
2.00	3.36	1.62
1.93	3.36	1.65
2.14	3.47	1.65
2.05	3.36	1.66
2.08	3.42	1.66
2.14	3.24	1.56
Av. 2.08	3.34	1.61
Av. % deviation 2.4	2.7	1.8

Table III. Determination of Silver and Bismuth in Lead

Silver %	Deviation %	Bismuth %	Deviation %
0.0056	0.0001	0.0315	0.0002
0.0057	0.0002	0.0315	0.0002
0.0054	0.0001	0.0310	0.0007
0.0055	0.0000	0.0315	0.0002
0.0051	0.0004	(0.0280)	...
0.0055	0.0000	0.0315	0.0002
0.0055	0.0000	0.0310	0.0007
0.0055	0.0000	0.0335	0.0018
0.0055	0.0000	0.0315	0.0002
0.0056	0.0001	0.0310	0.0007
0.0055	0.0000	0.0315	0.0002
0.0057	0.0002	0.0335	0.0018
Av. 0.0055	0.000092	0.0317	0.0006

Average deviation for Ag 1.67%; Bi 1.9%.
Maximum deviation for Ag 7.3%; Bi 5.7%

No attempt was made to obtain the highest possible precision by averaging the results of several photometric readings for each line, or to separate the errors in photometry from those inherent in the photographic process. Rather, it was sought to maintain the conditions of a routine analysis, so that the results may be taken as typical of ordinary work. In the wide density range covered, 0.1 to 1.6, it was found that average deviations of 2 to 3 per cent may be expected, with occasional maximum deviations as great as 5 to 7 per cent.

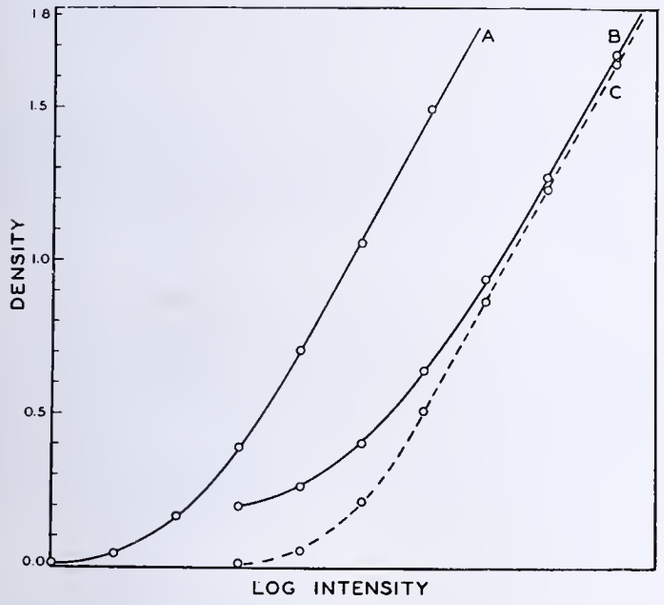

Figure 3. Effect of Background on Plate Calibration

The precision obtained in the analysis of homogeneous metal samples is comparable to that of the preceding experiment. This is shown by the data of Table III, obtained by Tlapa (*13*) for silver and bismuth in lead. The excitation source was a high-voltage spark. Sawyer and Vincent (*9*) obtain results of about the same precision in the analysis of sheet steel, using an alternating current arc source. Numerous other results of comparable precision might be cited. It would seem, therefore, that under favorable excitation conditions the limiting factor in the accuracy of spectrochemical analyses is the photographic process. This is not true, however, for most of the determinations that are made by means of a carbon supporting electrode. There a mean deviation of 5 per cent in a series of duplicate determinations is good and often it is not possible to obtain precision better than 10 per cent. In such analyses the limiting factor appears to be unequal vaporization and excitation of the constituents of the sample, and only a small portion of the error may be attributed to the errors of the photographic process.

Effect of Background

When a spectrum line lies in a region of heavy background the density value obtained is high, and a correction must be applied for the effect of the background. This correction cannot be obtained by subtraction of the background density from the total density or by the equivalent procedure of making the I_o reading in the background region. Rather, it is necessary to subtract the background intensity from the total intensity. The method for doing this is shown in Figure 1, by the dashed lines. A total density of 1.37 corresponds to a relative intensity of 30.8. The background density of 0.19 corresponds to a relative intensity of 2.4. Subtraction gives the corrected line intensity of 28.4. If for the same values the correction is made by subtraction of densities, a corrected line intensity value of 22 is obtained, which is too low by about 25 per cent.

The validity of the method for making background corrections was tested in the following experiment:

Two seven-step mercury arc spectra were photographed on a plate. On one of the spectra was superimposed a heavy background by re-exposing that portion of the plate to the spectrum from a carbon arc. Intensity ratios were determined for the various steps of the 2652–2654 Å. lines for the spectrum without background, for the uncorrected spectrum with background, and for the corrected spectrum with background. The results are shown in Table IV. After correction the intensity ratios for the various steps are reasonably good, but if the uncorrected ratios are used the values for the lowest exposure steps are in error by as much as 40 to 50 per cent. Data for the same plate are shown in the H. & D. curves of Figure 3. Curve A is for the spectrum which has no background, curve B for the one with background. At high intensities the effect of background is not great, but in the lower exposure steps the measured density is chiefly that of the background. Curve C shows corrected line densities, and is in good agreement with curve A.

Table IV. Effect of Background

Step	Intensity Ratio: Without background	With background	Corrected
1	2.14	1.85	2.00
2	2.07	1.84	1.86
3	2.06	1.61	1.98
4	2.08	1.40	1.87
5	2.03	1.22	1.83
6	..	1.12	1.94

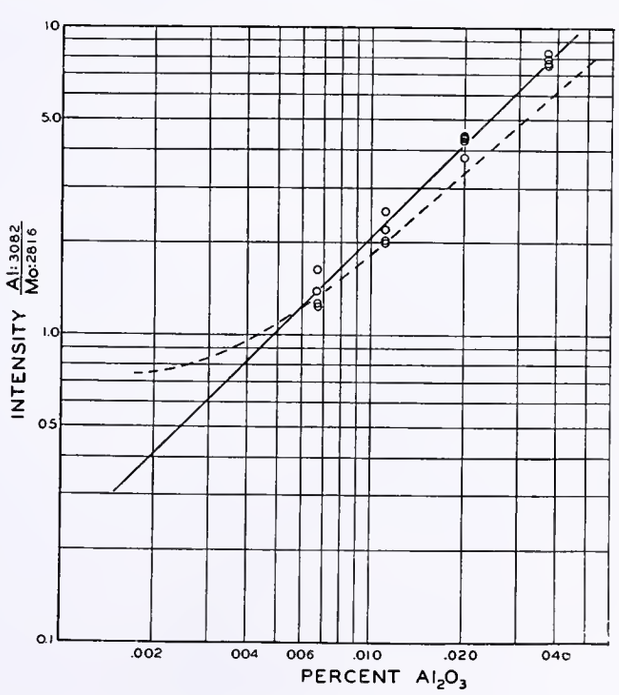

Figure 4. Effect of Background on Aluminum Working Curve

The effect of background in analytical work is shown in the working curves of Figure 4. The heavy line gives a working curve for the aluminum 3082 Å. line, made with the alternating current arc. The sample was a 3.6 *M* solution of sodium hydroxide to which known amounts of aluminum had been added. (All concentrations are based on the weight of dry sodium hydroxide.) Molybdenum 2816 Å. was used as internal standard line. The circles and the straight line give the intensity ratios corrected for background and for the residual amount of aluminum in the matrix. The dashed curve gives the intensity ratios obtained if the background correction is not applied; the concentration values have been corrected for the residuum. At high concentrations the two curves are nearly parallel to one another, but at low concentrations the uncorrected values indicate concentrations several hundred per cent in error. In fact, the curvature of the uncorrected line is like that which one obtains when the matrix holds a considerable residuum of the material sought in the analysis, and were the background correction not applied one might overcorrect for the amount of residuum.

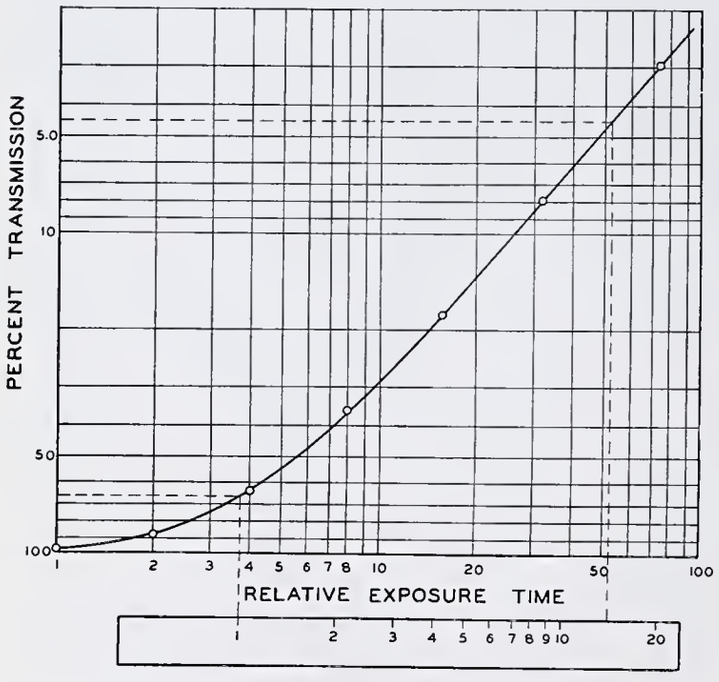

FIGURE 5. GRAPHICAL COMPUTATION OF RELATIVE INTENSITY
Log-log paper is used, so that photometer readings can be plotted without conversion to density values

It is obvious from these results that the plate calibration curve must be made from a background-free spectrum, for unless the true calibration curve is known down to low density values it is not possible to make accurate background corrections. It is, therefore, not a good practice to make the calibration curve from a stepped exposure for analysis samples, because such spectra usually contain a rather heavy background, especially if a carbon supporting electrode is employed. Some workers use no separate plate calibration curve but make all exposures with a stepped sector and plot H. & D. curves for every line. The concentration is then determined from the linear separation on the log exposure axis of the internal standard and analysis lines. This practice partially eliminates the effect of background, but leads to some error for faint lines because the measured density in such cases is largely that of the background, as can be seen from curve B of Figure 3.

Treatment of Experimental Data

The microphotometer data obtained from spectra are in the form of galvanometer deflections for clear plate and for spectral lines. From these readings one obtains intensity ratios for internal standard and analysis lines. The labor of this computation may be materially lightened by the proper choice of method.

It is generally advantageous to plot the plate calibration curve on a density–log intensity basis, when background corrections are not needed, because the curve obtained has a long linear portion. It is not necessary, however, actually to convert galvanometer readings into densities. If the photometer light intensity is so adjusted that the reading for the clear plate is 100, the readings obtained for lines are the percentage transmission values. These may be plotted *vs.* relative intensities by use of log-log paper, as shown in Figure 5. When background corrections are needed, however, it is advantageous to plot density *vs.* intensity. Here semilog paper can be used, with galvanometer readings on the log scale as shown in Figure 5 but with intensity values on a linear scale as in Figure 1.

Calculating boards of the type described by Owens (*8*) are advantageously used with either type of plot. The method for using the board to obtain intensity ratios when background corrections are not necessary is shown in Figure 5 by the dotted lines. The sliding scale at the bottom, graduated to agree with the x-axis, gives the intensity ratio of the two lines of the indicated densities. When background corrections are necessary one cannot directly obtain intensity ratios in a single operation. Rather, the board is used to make the background corrections, as indicated by the dotted lines of Figure 1, and the ratios of the corrected intensities of internal standard and analysis lines are determined in a separate slide rule operation.

Working Curves

Every spectrographic analysis is based upon a comparison of the intensity values obtained from an analysis sample with the values obtained from samples of known composition. The working curve, which relates functions of intensity and concentration, has in the past been expressed in many different ways; recently the practice has become fairly standardized, since today nearly everyone uses an internal standard whenever possible.

The relation between the intensity of light emitted within a source and the concentration of the emitting atom is linear. That is,

$$i = kC$$

or if an internal standard is used,

$$i_A/i_S = kC_A$$

where i_A and i_S are, respectively, the intensities of analysis and internal standard lines and C_A is the concentration of the constituent to be determined.

Unfortunately for the analyst, the intensity of light emitted from a source is not always a linear function of the concentration; some of the emitted light is often readsorbed in colder outer layers of the source. When such absorption occurs the relation between concentration and intensity is given by an empirical equation of the form

$$i_A/i_S = kC^n$$

The mechanism of light absorption is doubtless complicated, since the light traverses outer gaseous layers of various temperatures before it can be detected; a simple Beer's law absorption will not lead to the empirical intensity-concentra-

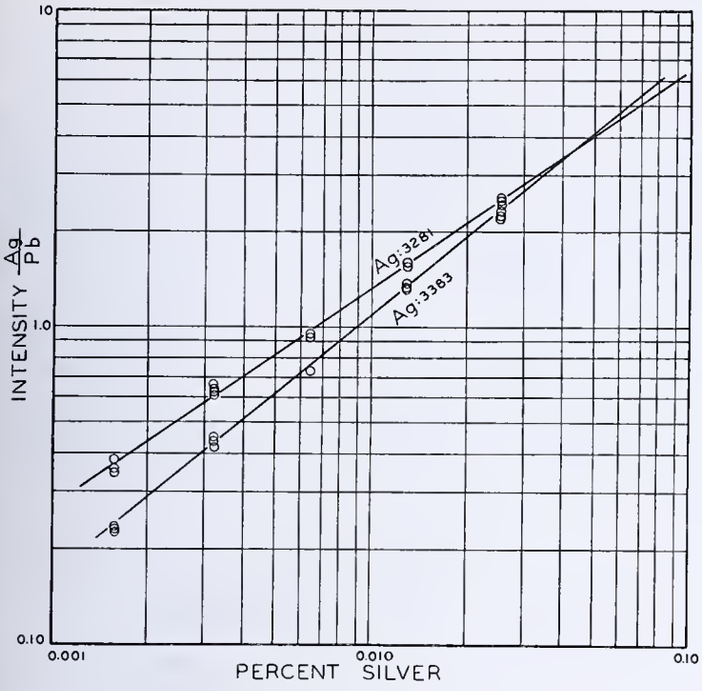

FIGURE 6. WORKING CURVES FOR SILVER 3281 AND 3383 Å. LINES

Illustrating effect of self-absorption on slope of intensity-concentration function

tion function given above. Consequently it cannot safely be assumed that this relation will hold in all cases. In the writers' experience, however, no case has been found which was not covered by one of the equations given above.

Regardless of whether or not there is self-absorption, a plot of log intensity ratio *vs.* log concentration will give a straight line of slope n. The value of n is unity when there is no absorption (as in Figure 4), less than unity when there is absorption (*2*). Thus, when there is no absorption one may obtain a straight line by plotting either intensity ratio *vs.* concentration or log intensity ratio *vs.* log concentration. The writers know of only two examples of such nonabsorbed lines which have been used for analytical purposes—namely, aluminum 3082 and silicon 2816 Å. When there is absorption only the log intensity–log concentration plot will give a straight line; consequently this form of plot is the one generally used.

The value of n varies, as one might expect, with different lines for the same element. An example of this is shown in Figure 6, which gives Tlapa's (*13*) results for the two silver lines 3281 and 3383 Å. At low concentrations the 3281 Å. line is the stronger, but at high concentrations this line is so highly absorbed that the 3383 Å. line appears to be the stronger. Because of this effect estimates of relative line intensities for a given element may be misleading because the apparent intensity may depend upon the concentration.

Working curves are usually prepared from spectral data for synthetic mixtures containing the matrix element and added amounts of the various constituents sought in the analysis. It often happens that the matrix material contains a residuum of unknown amount of some constituents sought in the analysis; in such cases all apparent concentration values are in error by a constant amount. When this happens one may from the spectral results make a determination of the amount of the residuum (*3*). This is done by a series of successive approximations.

First, a working curve plot is made of log intensity ratio *vs.* log apparent concentration. When a residuum is present the line will tend to become parallel to the concentration axis at low concentrations. A straight line is drawn through the points for the higher concentrations and the amount of the residuum is estimated by the magnitude of the departure of the lower concentration points from this line. Next, all concentrations are corrected by adding the estimated residual value, and the process is repeated. Usually two or three approximations will suffice to give a value which will bring all points onto a straight line. Finally, the correctness of the estimation is tested by using the working curve so obtained to determine spectrographically the amount of residuum in the pure matrix material.

The value so obtained should agree with the value obtained by means of the successive approximations. When this does not happen it is indication that some other factor, such as background, must be taken into account. For example, were one to attempt a residuum correction for the uncorrected aluminum working curve of Figure 4, the value obtained would be greatly in error because of the effect of the background.

Whenever one is fortunate enough to work with a nonabsorbed line, the determination of the residuum can be done in a single operation. A working curve is plotted as intensity ratio *vs.* apparent concentration, and a straight line is drawn through the points. Now, another parallel straight line is drawn through the origin; the displacement of the two lines on the concentration axis gives directly the amount of the residuum.

After a working curve has been accurately determined, the final form in which it is used depends upon whether background corrections are needed in the analytical spectra. No generalization can be made about this. When a carbon supporting electrode is employed it is usually advantageous to correct for background but in the analysis of metal samples by a spark source the background is often negligibly small. When background corrections are not essential it is advantageous to put concentration values directly on the slide of the calculating board instead of intensity ratios (this is facilitated by the linear relation between log intensity ratio and log concentration). When background corrections must be used it is, of course, impossible to obtain concentration values from photometer readings in a single operation, because of the necessity for subtraction of intensities. In this case the working curve is usually plotted as in Figure 4, and concentration values are determined from intensity ratios by means of this plot.

Internal Standards

Internal standards are used in practically all spectrographic analyses. The purpose of the standard is to permit the analyst to make corrections for unequal vaporization and excitation from sample to sample. None of the electrical sources used will give absolute reproducibility—that is, if a series of exposures is made for separate portions of a given sample, the line densities of the spectra obtained will vary over a considerable range. The chief reason for this lies in the fact that one cannot accurately control the rate of vaporization of the sample material. During the course of an exposure the temperature of the source changes as the vapor atmosphere changes. Therefore, the probability of excitation for a vaporized atom will change from time to time, since this probability depends upon the temperature of the source. The material used for internal standard should then possess the following characteristics:

The internal standard line should have the same excitation voltage as the analysis line, so that changes in temperature will affect both lines in the same manner.

The internal standard element should be vaporized at the same rate as the constituent sought in the analysis.

The internal standard should be present at such concentration that the line density is about the mean density found for analysis

lines in the concentration range investigated, so that the lines to be compared will be as nearly as possible of the same density.

The internal standard must be homogeneously mixed with the analysis sample.

In practice it is not always possible to attain all these conditions, particularly the first two, and the accuracy of the analysis depends upon the extent to which they are fulfilled. Best results are obtained in analyses made by spark excitation of homogeneous metal samples, where the vaporization rate and the source temperature may be held at a very constant level. Here almost any matrix element line of proper density and wave length can be used as standard. More difficulty is encountered in the analysis of solutions with the alternating current arc, because the choice of standard is often limited by the composition of the sample and by solubility in the sample solution. In some cases a line of the matrix material will serve as standard, but in others the standard must be an added constituent.

In selecting a standard for an analysis of this type, one first examines a spectrum to determine the impurity elements present, for no element present as impurity can be used as standard. Next, a wave-length table is examined for elements not present which give strong lines near the lines to be used for the analysis. From these, one selects those elements which have compounds soluble in the sample solution. Test spectra are made, with varying amounts of these elements added to the analysis sample, in order to select proper concentration ranges for the standard and to ascertain whether the spectrum of the standard has any lines which interfere with the analysis. This procedure will often limit the choice of standard to a few elements.

Among the most widely used internal standards are molybdenum and cobalt, because they are of infrequent occurrence, they provide many suitable lines in the ultraviolet region, and they vaporize from the source at an average rate. Zinc, cadmium, mercury, and other elements whose salts are very volatile are seldom used as standards because they do not vaporize at anything near the rates of those substances usually sought in analyses. In the analysis of powdered samples, for which the direct current arc must be used, it is often debatable whether the internal standard method offers any advantages. The source may change greatly in temperature during the arcing of a sample and unless the internal standard vaporizes uniformly with the sample it will not serve its purpose. Further, proper mixing of the internal standard with the sample entails laborious grinding. Because of these difficulties Slavin recommends (*10*) that the standard be omitted; he prefers to arc weighed portions of sample to complete vaporization.

Some have used background intensity in lieu of internal standard. This procedure is valid only in those cases where the background is excited concurrently with, but independently of, the analytical spectrum. An example of this is in Lundegardh's work (*6*) with flame excitation. Here the background comes from the flame itself and changes in excitation condition apparently are manifested by changes in background. In electrical excitation the background is due to cyanogen bands (when carbon electrodes are used), to incandescent particles of sample, to band spectra from sample material, and to nitrogen bands. All of these, except band spectra of the sample, may vary fortuitously from one sample to another.

Microphotometers

Nonrecording, photoelectric-type microphotometers are almost universally used in spectrochemical analytical work. A microphotometer of this type should have the following characteristics:

Freedom from stray light effects. If stray light reaches the photocell all transmission readings are too high and the error in line density measurements is increased.

Sensitivity. The instrument should be sufficiently sensitive to give full-scale deflection with a slit which is not over one third the width of the line to be measured (or of the projected image of the line).

Stability. The scale reading should remain constant within 1 per cent over a period of at least 10 minutes, so that the I_o reading need not be taken at frequent intervals.

Rapid response. The galvanometer period should be so low that a line density can be measured within a 15-second period after the plate is adjusted to align the line with the slit.

Ease of plate adjustment. The construction should be such that the operator can quickly and accurately align the spectrum line with the slit.

Adjustable sensitivity. There should be some convenient control which permits the operator to adjust the I_o reading to a scale deflection of 100, so that the readings for lines give percentage transmissions directly.

Linearity of response. The galvanometer deflection should be proportional to the transmitted light intensity throughout the range.

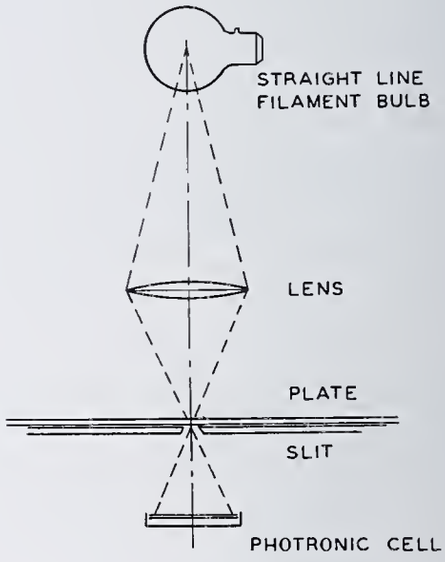

FIGURE 7. OPTICAL SYSTEM OF PHOTOMETER

An instrument which fulfills these criteria was constructed according to the design shown in Figure 7.

The essential features are a low-power light source that gives constant intensity and a slit arrangement that minimizes stray light. The plate is mounted on a screw-driven carriage, emulsion side down. The slit, usually measuring about 1.5 × 0.015 mm., is mounted beneath the plate, with a clearance of about 0.2 mm.

The source is a straight-line, coiled-filament headlight bulb operated at 6 volts and 5 amperes by a constant-power transformer. A reduced image of the filament is focused at the slit. A photovoltaic cell is used in conjunction with a sensitive galvanometer to measure light intensity. The maximum photoelectric current is between 10^{-5} and 10^{-6} ampere. Sensitivity is controlled by a resistance in series with the light, after a rough adjustment is made by varying the dimensions of the slit to bring the deflection to the approximate value desired.

The plate is aligned by means of a mark which runs perpendicular to the center of the slit opening, on the slit jaws. After the spectrum is aligned with the slit the operator slowly moves the carriage by a lead screw, so that the line is made to travel across the slit, while he observes the galvanometer deflection. With a little practice it is easy to coordinate the rate of movement with the galvanometer period, so that the point of maximum density is not overrun. The speed is somewhat better than a line per minute, including a reading of the background transmission.

In all respects except convenience of alignment and speed of operation this instrument compares favorably with the commercially built instruments which have been available for tests. It is very stable, the I_o value remaining constant for long periods after an initial warming up. Because of the low light intensities used and the isolation of the cell from the heat

of the lamp, the cell current is very steady and shows no appreciable drift with time. The amount of stray light entering the slit is negligibly small, as shown by the fact that plate calibration curves remain linear up to densities well above 2. In this respect this instrument is better than all but one of the commercially built instruments tested. The response is uniform over the entire scale. This was tested by a series of measurements of the relative transmissions of two screens, with the slit adjusted to give total deflections varying from 5 to 100 cm. The ratio remained constant over the whole range. Reproducibility is good; remeasurement of a whole plate seldom gives deviations greater than 0.01 density unit except for lines of density greater than 1.5, where the error in reading the deflection is large.

Before this instrument was designed attempts were made to build a projection-type photometer by projecting the spectrum image onto a screen at X10 enlargement and placing the slit and photocell behind the screen. This type of instrument was abandoned because of the stray light effects which could not be eliminated. It was shown that the stray light originates in the light scattered from the immediate vicinity of a line, since the instrument gave correct density readings for wide test spots but readings as much as 50 per cent in error for dark lines.

Summary

A study has been made of the photometric methods used in spectrochemical analyses and the procedures which have been found to give optimum results are listed. Included in this study are the methods for plate calibration, the variations in plate gamma with wave length, the effect of background and methods for correcting, and the accuracy obtained in photometric measurements of intensity ratios. Working curves and the factors governing the selection of an internal standard are discussed. Graphical computation methods are described. Criteria for evaluation of a photometer are given and the construction of a satisfactory instrument is described.

Acknowledgment

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Preparation of a Solution of *o*-Phthalaldehyde for Use as a Glycine Reagent

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GLYCINE is determined colorimetrically by condensing with *o*-phthalaldehyde according to the Patton (*3*) and Klein and Linser (*1*) modifications of Zimmermann's method (*6*). The aldehyde cannot be purchased and has generally been prepared from *o*-xylal bromide (*2*), which can no longer be obtained on the market. The authors attempted to prepare it by the bromination of *o*-xylene, but with very little success. Their efforts were then directed to the preparation of the *o*-phthalaldehyde reagent from *o*-xylene by modification of the method of Thiele (*4*, *5*).

A 1-liter three-necked balloon flask is fitted with a calcium chloride drying tube, a dropping funnel, and a stirrer. The flask is cooled in an ice bath while 150 ml. of acetic anhydride, 10 grams of *o*-xylene, and 8 ml. of concentrated sulfuric acid are introduced. Twenty-six grams of chromium trioxide are dissolved in a mixture of 50 ml. of acetic anhydride and 60 ml. of glacial acetic acid; this solution is added drop by drop from the funnel with stirring. The reaction flask is kept in the ice bath for 4 or 5 hours, during which time the mixture is continuously stirred. The contents are poured into a 1-liter beaker one fourth filled with cracked ice and placed in an icebox over night. A yellow oily scum collects on the surface. The whole is extracted several times with ether, and the combined ether extracts are washed with water to remove the last traces of chromous compounds and dried over anhydrous sodium sulfate. The ether is distilled off on a steam bath and the last of the ether is removed by a vacuum. All attempts to remove the acetic acid with carbonate at this stage resulted in a loss of the active compound.

The residue, consisting of acetic acid plus phthalaldehyde tetraacetate, is treated with 50 ml. of 10 per cent sulfuric acid and steam-distilled as long as the distillate gives a blue color upon adding a drop of ammonium hydroxide and acidifying with acetic acid. About 500 ml. of the glycine reagent result and should be stored in a dark bottle.

The reagent is now ready for use with either a protein hydrolyzate or a solution containing glycine (*2*), except that it should be brought to a pH of 7.4 to 7.8 immediately before use. Attempts to isolate the solid aldehyde resulted in unstable oily crystals; this phase was not pursued further.

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Woburn Iodine Absorption Method

A Measure of Total Unsaturation in the Presence of Conjugated Double Bonds

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The iodine reagents now in use react incompletely with substances containing conjugated double bonds. The methods recommended for determination of the total unsaturation of conjugated oils and fatty acids are complicated and have not found general use in industrial analysis.

Hanus solution, when used in large excess, measures the total unsaturation of dehydrated castor oil, but this procedure is unsuitable for other conjugated oils and fatty acids. By employing iodine bromide solutions of up to twice the concentration used in the Hanus method, a simple procedure is evolved for both conjugated and nonconjugated substances.

Fatty acids with conjugated double bonds, made by an isomerization process from natural fatty acids, give low iodine values with the standard methods. With the proposed method, their iodine values are identical with those of the nonconjugated fatty acids from which they were made. With dehydrated castor oil, constant values result over a wide range of excess of reagent.

With tung oil, a reproducible value of about 225 is obtained if temperature, excess, and time of contact are kept within certain limits. The effect of changes in working conditions on the value for the total unsaturation of tung oil somewhat resembles the effect of similar changes in determination of the Wijs iodine value of this oil.

Theoretical values are obtained with beta-eleostearic acid, 9,11-linoleic acid, and blends of the latter with nonconjugated fatty acids, if proper conditions are used. Values for oiticica oil and for nonconjugated oils and acids are listed.

NUMEROUS halogen-absorption methods are available for measuring the unsaturation of oils and fatty acids. The majority of the customary methods are satisfactory with oils containing only single ethylenic linkages and isolated double-bond systems. They are known to fail, however, with oils containing conjugated double bonds, such as tung oil and oiticica oil.

Gelber and Boeseken (*8*) found that the Wijs iodine reagent adds quickly to one of two conjugated double bonds in 9,11-linoleic acid, or two out of three conjugated double bonds in eleostearic acid, while the remaining double bond is saturated only after contact of 2 to 6 days. Ordinarily, the Wijs method will give a value with tung oil which is somewhat above the partial saturation point, where two out of three double bonds of eleostearic acid have taken up halogen. van Loon (*23*) termed the resulting value the "apparent iodine number". Various investigators have studied the effect of temperature, time, and excess of reagent on the apparent iodine value of tung oil and other oils with conjugated double bonds (*10*). As Gelber and Boeseken (*8*) and van Loon (*23, 24*) have shown, it is possible to determine the true total iodine value of conjugated oils with Wijs solution by extending the time of contact up to one week and applying up to 700 per cent excess of reagent. As a practical method of analysis such a procedure is inadequate because of its slowness.

The von Huebl iodine method, commonly used before introduction of the Wijs, Hanus, and other more recent methods, was applied to tung oil by Kreikenbaum (*20*), who found that it gave uniform results from 169 to 171. Kubelka and collaborators (*21*) showed that the methods of Margosches and Rosenmund-Kuhnhenn similarly give values for tung oil approximating the two-thirds or partial saturation point. None of these methods (and this is true of others as well) is a measure of the total unsaturation—i. e., all the double bonds present, individual, isolated, or conjugated.

With the Hanus method, the situation is somewhat different. The results obtained on tung oil vary even more than most of the others with time, temperature, and sample weight (*11, 32*). Kreikenbaum (*20*) found Hanus values on tung oil varying all the way from 188.9 to 210.8 and considered the Hanus method "inapplicable to Chinese wood oil". Other investigators had similar results. Wiernik (*43*) obtained a Hanus iodine value of 205 on a sample of tung oil, and the same sample when checked by a standard laboratory yielded values of from 139 to 153.7. Wiernik, therefore, concluded that sometimes 2 and sometimes 3 double bonds of eleostearic acid react with iodine bromide solution. The American Chemical Society and American Oil Chemists' Society Committee on Analysis of Commercial Fats and Oils found that the Hanus reagent is entirely unsuitable for tung oil, since extremely high and irregular results are obtained (*1*). Kubelka and co-workers (*21*) studied the effect of sample weight on the Hanus iodine value of tung oil and obtained a curve including values from 122.69 to 163.56 for sample weights of 0.1210 to 0.2542 gram. When the sample weight was kept constant, however, and the time of contact was varied from 15 minutes to 24 hours, values from 163.05 to 276.03 resulted. It was concluded that with Hanus reagent the true iodine value of tung oil was reached after 3 hours, when the absorption had attained a value of 243.42.

Ralls (*31*) modified the Hanus value to a microprocedure, in which iodine bromide solution of one half the strength ordinarily used is employed at ice-bath temperatures. While this procedure was apparently not tried on substances containing conjugated double bonds, it did give theoretical values on such substances as undecylenic acid and cholesterol, which ordinarily do not yield theoretical iodine values. However, this method gave erroneous values, in some cases almost 100 per cent too high, with castor oil, which does not afford difficulties with the common iodine procedures.

The method of Volmar and Samdahl (*42*) also was found applicable to substances with which other procedures fail, such as hexadecene and cinnamic acid, but no report on the use of this method with oils containing conjugated double bonds could be found. The method is slow, involves bromination at 0° to −10° C., isolation of the addition compound, and determination of the bromine by the Charpentier-Volhardt method.

Special Procedures for Compounds with Conjugated Double Bonds

There is, however, a group of halogen-absorption methods which is able to measure all double bonds, including those in the conjugated position.

Kaufmann (*16*) uses ultraviolet light to catalyze the reaction of bromine with the third double bond of eleostearic acid. With others, bromine vapor is allowed to act on a thin film of the substance to be tested. This procedure was first employed by Becker (*2*) and later improved by Rossmann (*35*). Modifications have been suggested by Sabalitschka and Dietrich (*36*), Toms (*39*), Boeseken and Pols (*4*), Brocklesby and Harding (*6*), and others, but have not found extensive use. The bromine absorption is determined gravimetrically and, because of the small sample weights used, a microbalance is required.

Some investigators have called attention to the difficulty of re-

moving the excess bromine vapor from the film (*4*). However, Rossmann (*33*) devised a procedure by which the excess bromine can be titrated, making superfluous the driving off of the excess bromine vapor from the film and the final weighing of the films to constant weight. He obtained theoretical values for alpha- and beta-eleostearic acids, 9,11-linoleic methyl ester, and various other substances with active and inactive double bonds. Later (*34*), however, he found that the volumetric determination fails in the presence of polymerized and oxidized oils, hydroxy acids, and certain other substances. Castor oil with an iodine value of 82 gave bromine-iodine values of 101 to 119 for 5 to 30 minutes' contact. Boeseken and Pols (*4*) believe the bromine-vapor methods to be unsuitable in the presence of hydroxyl groups and found that bromine attacks the hydroxyl radical in ricinoleic acid in addition to saturating the double bond present.

A different bromination procedure, in which bromine is formed in the presence of the fat to be analyzed by the action of potassium bromate on acidified potassium bromide solution, was proposed by Vaupel (*41*). By varying the excess of bromine and the time of reaction, Vaupel obtained so-called primary, secondary, and tertiary bromine values. These values have, however, no relationship to the total unsaturation of conjugated fatty acids and oils, as indicated by the fact that the tertiary bromine value of tung oil, 205, was found to be lower than that of linseed oil, 240 (*40*).

Among the methods not employing halogen, oxidation with peracetic acid was recommended by Smit (*37*) for oils with nonconjugated double bonds only; however, Knowles, Lawson, and McQuillen (*19*), using a similar reagent, claim better results for total unsaturation of conjugated systems than with the Wijs method.

Quantitative hydrogenation has been successfully applied to beta-eleostearin and tung oil by Jordan (*15*). This method is considered to yield accurate values for total unsaturation, as there is no possibility of substitution or other side reactions which frequently complicate the bromine-addition procedures.

Table I shows the iodine values for total unsaturation of tung oil obtained by various investigators, using the methods discussed.

None of these proposed methods for the determination of total unsaturation of oils and fatty acids with conjugated double bonds compares in simplicity with the ordinary volumetric iodine-absorption procedures, such as the Wijs or Hanus method. The modified Wijs procedure, used by Gelber and Boeseken or by van Loon, requires an undue length of time. The need of an ultraviolet lamp complicates the method of Kaufmann, and the use of this procedure has not frequently been reported. Hydrogenation requires special equipment and the same is true, although to a lesser degree, of the bromine vapor method. Bolton and Williams (*5*) say of the latter that it "requires very careful manipulation, particularly with regard to the weighing of the brominated compound".

Table I. Iodine Values for Total Unsaturation of Tung Oil Obtained by Various Authors

Date	Authors	Method	Iodine Values
1928	Toms (*39*)	Bromine-vapor	217.0–232.4
1929	Gelber, and Boeseken (*8*)	Modified Wijs	240–250
1930	van Loon (*24*)	Modified Wijs	220–227
1930	Bolton and Williams (*5*)	Bromine-vapor	223.8–229.0
1933	Lévy (*22*)	Bromine-vapor	228–234
1934	Jordan (*15*)	Hydrogenation	214–226
1935	Rossmann (*34*)	Bromine-vapor gravimetric	238–261
		Bromine-vapor volumetric	245–266
1935	Boeseken and Pols (*4*)	Bromine-vapor	244.4–249.5
1936	Brocklesby and Harding (*6*)	Bromine-vapor	227.7
		Hydrogenation	225.5 (corrected, 226.5)
1940	Knowles, Lawson, and McQuillen (*19*)	Oxidation	208

Need for New Method

There is a steadily rising need for a simple method of determining total unsaturation, which is suitable for routine work, requires no special apparatus, and is applicable to both conjugated and nonconjugated substances. With tung oil, the apparent iodine value, determined by the present standard methods, is so little representative of its quality and performance that numerous practical tests, such as gelation tests, are commonly employed in its evaluation. The use of the partial rather than the apparent iodine value has been suggested (*5*, *27*). This is a true constant rather than an arbitrary value, and eliminates to a great extent the effect of varying sample weights and other details of operation.

The use of the true total iodine value of tung oil, on the other hand, would have at least three further practical advantages.

1. Tung oil, with the highest total unsaturation of all drying oils, would be moved to the top of the list where it belongs by virtue of its drying speed. Conjugated double bonds, which are the most effective for fast drying, will no more appear as a discount in the iodine value of the oil.
2. The common adulterants with iodine values near the apparent iodine number of tung oil will have a considerable effect on the total iodine value and become easier of detection. Similarly, changes in composition due to oxidation or polymerization may be observed by a decrease in total iodine value, whereas the partial and the apparent iodine values are little affected and may even increase during polymerization (*38*).
3. The total iodine value when determined in combination with the partial iodine value may serve as a measure of the amount of conjugated double bonds present.

In the case of oiticica oil, it has not been possible to date to find any reproducible iodine value (*12*). Kaufmann and Baltes (*17*) ascribe the difficulties to the presence of the keto group which "interferes so much with the iodine test that the analyst finds it impossible to determine any iodine number at all". However, van Loon (*25*) reports that the Wijs iodine value of both oiticica and po-yoak oil with large excess of reagent became constant after one week, as in the case of tung oil. A practical method of determining the total unsaturation, if applicable to oiticica oil, may greatly facilitate the analysis and characterization of this oil.

The introduction of dehydrated castor oil in the United States during the last several years has increased the need for a practical method of determining total unsaturation. Priest and von Mikusch (*30*) discussed the inadequacy of using a partial or apparent iodine value in the comparison of either dehydrating processes or the resulting oils.

Finally, the shortage of tung oil and the search for new oils, which possess conjugated double bonds and the physical properties associated with them, has brought into existence other synthetic oils and fatty acids containing this particular double bond structure. In the development of these products, a method giving the total unsaturation has been indispensable, and such a method is also required for the proper analysis and characterization of the finished products. The manufacturing process of the products here referred to—for example, Conjusoy and Conjulin fatty acids (*44*)—includes the isomerization or shifting of the isolated double bonds of natural fatty acids. Since the ordinary iodine reagents measure all isolated but only a portion of the conjugated double bonds, the apparent iodine value drops during isomerization. On the other hand, the total unsaturation will stay the same if other reactions such as polymerization or oxidation are excluded. Where polymerization takes place simultaneously with isomerization, this will be shown by a decrease in the total iodine value. Similarly, in the finished product the total iodine value is of greatest significance; its use in combination with the partial iodine value for characterizing the composition of the fatty acid or oil will be discussed in another paper.

Table II. Hanus Iodine Values of Conjugated Linseed Fatty Acids

(Theoretical total iodine value, 185.9)

Weight of Sample	Excess of Halogen	Iodine Value Found	Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%		*Gram*	%	
A, 24 Ml. of Hanus Solution, 1 Hour			B, 49 Ml. of Hanus Solution, 2 Hours		
0.0565	550	164.8	0.0533	1182	173.6
0.0589	538	161.2	0.0603	1030	174.2
0.0829	340	159.9	0.0864	674	177.2
0.0842	336	157.9	0.1110	512	174.6
0.0912	310	155.2	0.1672	339	161.5
0.0990	290	150.4	0.2102	269	153.2

Tests with Hanus Solution

It was observed in this laboratory (*27*) a few years ago that Hanus' iodine bromide solution, used in an excess of more than 400 per cent, yields a reproducible value with dehydrated castor oil which agrees with its total unsaturation calculated from the decrease in acetyl value during dehydration. Moore and Cranmer (*28*), who used this special Hanus method with all commercially available dehydrated castor oils, found that it had a reproducibility of one unit.

In order to determine the usefulness of this procedure for other oils containing conjugated double bonds, possibly in greater proportion, a sample of conjugated linseed fatty acids (*44*) was tested with Hanus solution in varying excess. The resulting values were inconsistent and, unlike dehydrated castor oil, depended on sample weight even when more than 400 per cent of reagent was present (Table II, A).

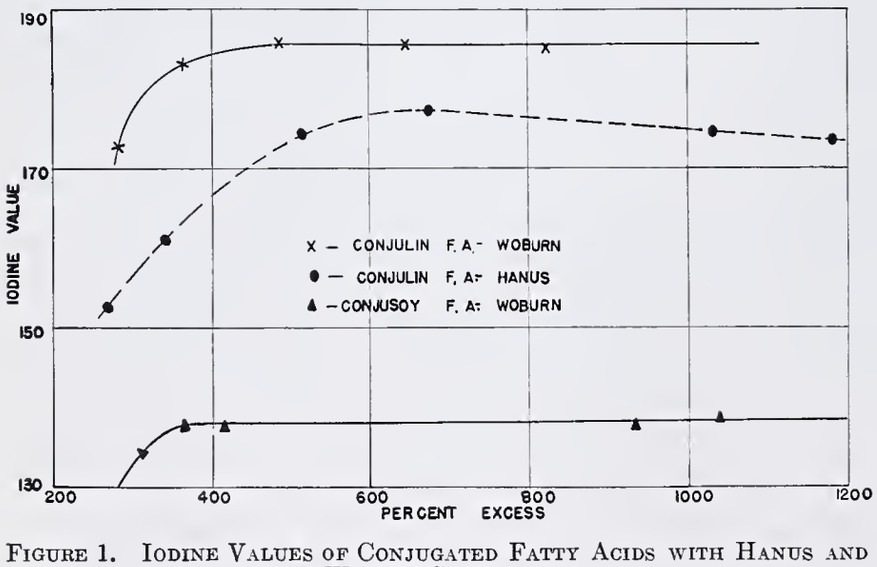

Figure 1. Iodine Values of Conjugated Fatty Acids with Hanus and Woburn Solutions

In order to supply a still larger excess of reagent, another series of iodine values was determined with varying sample weights, using approximately twice the ordinary amount of Hanus solution—i. e., 49 instead of 24 ml. The time of contact in this series was 2 hours.

The results are listed in Table II, B, and plotted in Figure 1 (broken line). They show that Hanus iodine solution, even when employed in an excess of up to 1200 per cent, results in low values which vary with the sample weight. The theoretical total iodine value listed is the iodine value of the linseed fatty acids before isomerization.

It was, however, thought likely that by suitable changes an iodine bromide reagent could be caused to react with all double bonds present, since this reagent had previously given the highest iodine values with oils containing conjugated double bonds of any of the halogen solutions employed.

Among the factors which might be changed to make the reaction more complete with Hanus solution are temperature, light, and time. An increase in temperature was not believed advisable because of the known predominance of side reactions, such as substitution and others at higher temperatures (*7*). Similar considerations hold for exposure to irradiation. An excessive extension of the time of contact also was undesirable because of the need for speed in industrial analysis. A 2-hour period had been found insufficient (Table II, B). A further prolongation of contact could be considered only if all other possibilities should fail.

Iodine Bromide Solutions of Higher Concentration. Changes in the excess of reagent already have been considered and found insufficient to ensure complete saturation. This left one other factor which might be modified—i. e., the concentration of halogen. A study of the extensive literature on iodine values reveals that this factor has not received much attention. Most investigations of the effect of concentration on the iodine number have been limited to increasing the volume of reagent used in a determination, or decreasing the sample weight. In the former case, the concentration of halogen, it is true, increases somewhat, provided the volume of solvent used with the sample is kept constant. In the latter case, for very small sample weight, the halogen concentration at the end of the reaction reaches a limiting value equal to its concentration at the beginning of the time of contact, while at the same time the concentration of the oil approaches zero. An increase in the concentration of both halogen and oil may be attained by reducing the volume of chloroform or carbon tetrachloride in which the sample is dissolved. The effect of increasing its volume in the Wijs method has been discussed by Keffler and Maiden (*18*) and tested in the analysis of tung oil in this laboratory (*27*). If the solvent for the sample is omitted altogether or reduced to a minimum, the concentration of reagent is increased in the ratio of 35 to 25, or by 40 per cent. This represents the maximum increase attainable for a given halogen solution.

In order to obtain a more substantial increase in the concentration of halogen, it is necessary to begin with a more concentrated reagent.

Hanus (*9*) prepared his solution by dissolving 20 grams of iodine bromide in 1 liter of acetic acid. His solution was, therefore, approximately 0.1934 *N* or 0.0967 molar. In the customary standard methods of preparing Hanus solution (*14*) this is made to contain 13.2 grams of iodine and the equivalent weight of bromine per liter and is, therefore, 0.208 *N* or 0.104 molar. The authors were unable to find references in the literature to the use of iodine bromide solutions of higher concentration, except in the method of Bellier (*3*), in which the dissolved sample is titrated directly with an iodine bromide solution containing mercuric chloride. One milliliter of Bellier's solution corresponds to 0.1 gram of iodine; the solution is, therefore, 0.788 *N*.

The data presented below show the action of 0.32 *N* to 0.40 *N* iodine bromide solutions in acetic acid. Directions for the preparation of the 0.32 *N* solution and details for carrying out the determinations are given at the end of this paper. The 0.40 *N* and other iodine bromide solutions were made in various ways; in principle they consisted of making a saturated solution of iodine in glacial acetic acid (empyreuma-free) with or without the aid of heat and adding, at room temperature, a solution of the equivalent amount of bromine in enough acetic acid to reduce the concentration to the desired normality. The solubility of iodine in acetic acid at 25° C. is 0.1025 mole of iodine, or 26.02 grams per liter (*13*). A saturated solution, therefore, contains 0.1 mole in 976 ml. and requires 24 ml. of bromine–acetic acid solution, containing 0.1 mole of bromine to make it 0.40 *N*.

TABLE III. TOTAL UNSATURATION OF CONJUGATED LINSEED FATTY ACIDS

(Theoretical iodine value 185.9, Wijs iodine value (400% excess) 152, concentration of IBr solution 0.40 *N*)

Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%	
0.0824	822	185.1
0.1016	648	185.9
0.1296	484	185.8
0.1653	363	183.5
0.2120	284	172.8

TABLE IV. TOTAL UNSATURATION OF CONJUGATED SOYBEAN FATTY ACIDS

(Theoretical total iodine value 138.0, Wijs iodine value (400% excess) 117, concentration of IBr solution 0.32 *N*)

Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%	
0.0643	1040	138.6
0.0713	934	137.9
0.1254	414	137.6
0.1588	365	137.9
0.1843	311	134.5

Isomerized Fatty Acids

The sample of conjugated linseed fatty acid which had given inconsistent and low results with Hanus solution (Table II) gave the values shown in Table III with the 0.4 *N* iodine bromide solution for 1 hour at 20° C.

The resulting values are independent of sample weight or excess of reagent if the latter is at least 450 per cent (Figure 1, top), and this value is identical with the iodine value of the fatty acids before isomerization.

A sample of conjugated soybean fatty acids, which in the unisomerized form had an iodine value of 138.0, was tested with 0.32 *N* solution (Woburn solution) with varying sample weights following Method A. The results again were constant over a large range of sample weights and corresponded to the theoretical value.

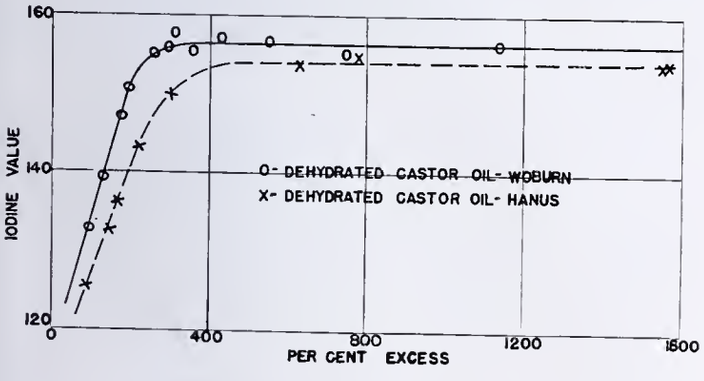

FIGURE 2. IODINE VALUES OF DEHYDRATED CASTOR OIL

DEHYDRATED CASTOR OIL. Samples of unbodied dehydrated castor oil (Isoline) of 1.4 poises viscosity (*F* on Gardner-Holdt scale) were tested by the new method, using varying sample weights. The theoretical total unsaturation of this oil is approximately 150 to 160, depending on the extent of dehydration (*30*). With a quantitative hydrogenation method, Muenzel (*29*) found that 1.5 grams of the oil take up 230 ml. of hydrogen at 15° C. and 740 mm. pressure, which corresponds to an iodine value of 160–161. A sample which had an apparent iodine value of 140 by the modified Wijs method with 400 per cent excess of reagent for 30 minutes at 20° C. gave constant values, within the experimental error averaging 156.4, with a 0.36 to 0.38 *N* iodine bromide solution applied in 300 to 1100 per cent excess (Table V).

Table V also shows the iodine values obtained with a similar solution on the same sample when the time of contact was varied from 5 to 60 minutes. These data indicate that over 90 per cent of the addition takes place in the first 5 minutes, over 95 per cent in the first 15 minutes, and that the constant region, corresponding to the horizontal section of the sample weight *vs.* iodine value curve (Figure 2, top) is reached between 45 and 60 minutes' contact. In this series the excess of reagent was at the lower limit required to obtain reproducible values in 1 hour. In the higher excess region constant values should be expected at shorter time of contact.

A different sample of unbodied dehydrated castor oil (Isoline), which had an apparent iodine value of 139.6 by the special Wijs method, gave Woburn values of 159.6 and 158.5 (average 159.1) with 0.4 *N* iodine bromide solution.

The Hanus iodine values which were found several years ago (*27*) on another sample of dehydrated castor oil have been plotted against excess of halogen in Figure 2 (broken line). Comparison of this curve with the new one shows that both solutions give reproducible values above a certain excess of reagent. However, with Hanus solution the minimum excess required is above 400 per cent, while the concentrated iodine bromide solution requires only 300 per cent. While in the modified Hanus method the effective range of sample weights is between 0.06 and 0.08 gram, samples weighing up to 0.16 gram are well within the region of constancy in the new method. The difference of 2 to 3 points between the constant portions of the two curves may be due to a difference in total unsaturation of the two oil samples.

TUNG OIL. Preliminary tests showed that the values obtained by Method A—i. e., 1 hour at 20° C.—could not be so well reproduced with tung oil as with the synthetic oils and fatty acids which contain both isolated and conjugated double bonds. Both excess of reagent and time of contact affected the results. When the time of contact was extended beyond 1 hour, the values increased, indicating that two reactions of different speeds were superimposed. In order to separate these reactions as much as possible, further determinations were made in an ice bath following the details of Method B, given below. The temperature of the reaction mixture under these conditions is 5° to 10° C. at the start but drops to 0° C. during the early stages of the reaction.

TABLE V[a]. TOTAL UNSATURATION OF DEHYDRATED CASTOR OIL

(Theoretica total iodine value 150–160, Wijs iodine value (400% excess) 140, concentration of IBr solution 0.36–0.38 *N*, temperature 20° C.)

Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%	
	(Time, 1 Hour)	
0.0627	1138	156.3
0.0903	747	155.3
0.1179	542	156.8
0.1476	412	157.0
0.1692	350	155.7
0.1857	307	157.2
0.1932	289	156.0
0.2107	258	155.4
0.2625	196	150.5
0.3008	164	147.2
0.3600	133	139.5
0.4479	97	133.0
	Excess of Halogen, 320 to 300 Per Cent)	
	(Time, Min.)	
0.2053	5	144.8
0.1917	15	152.5
0.2104	30	153.2
0.1924	45	154.9
0.1726	60	156.6

[a] Values determined by Victor Volk.

The following samples of tung oil, some characteristics of which are listed in Table VI, were used:

1. Old sample of unknown origin, stored without precaution against oxidation. Partial iodine value (*27*) 158.1.
2. Fresh drum lot of Chinese origin, analyzed immediately after sampling.
3. Florida tung oil, several years old, stored without precaution against oxidation.
4. Fresh sample of Chinese origin, through the courtesy of John R. Rea of New York.
5. Standard analyzed sample of American tung oil, cold-stored in well-filled containers; from National Paint, Varnish and Lacquer Association through the courtesy of H. A. Gardner. The characteristics supplied with this sample, other than those listed in Table VI, were:

Specific gravity at 15.5–15.0° C.	0.940
Saponification No.	193.0
Unsaponifiable matter, %	0.44
Refractive index at 25° C.	1.5170
Iodine No. (Wijs)	167.4

Table VII contains the values found on these five tung oil samples with varying conditions. The use of the ice bath as described in Method B is indicated by "0° C." in the column headed "temperature".

Table VI. Characteristics of Tung Oil Samples Tested

Sample No.	Origin	Viscosity (Gardner-Holdt)	Color (Hellige)	Acid Value	Browne Heat Test, Min.
1	Unknown	K-L	4L-4	6.4	10.75
2	Chinese	I-J	2L	4.5	11.75
3	American	U	2L	0.93	6.25
4	Chinese	J	3L-3	3.5	10.25
5	American	I-J	1-2L	0.9	11.5

Table VII. Iodine Values of Tung Oil

Normality of IBr Solution	Time *Hours*	Temperature ° *C.*	Excess %	Iodine Value Found
		Sample 1		
0.36	3	20	689	213.4
0.36	3	20	585	213.2
0.36	3	20	289	202.8
		Sample 2		
0.352	1	20	496 ± 2	224.2 ± 0.3
		Sample 3		
0.362	1	20	670	230.0
0.362	1	20	550	225.8
0.362	1	20	300	230.5
		Sample 4		
0.324	1	0		206.2
0.324	2.5	0	458 ± 58	216.4
0.324	4.2	0		220.6
0.324	6	0		215.8 ± 1.9
0.324	11.5	0	389 ± 53	222.0 ± 0.6
0.324	18	0		225.1 ± 1.9
0.400	1	0		219.9 ± 0.5
0.400	2.5	0		225.4
0.400	3	0	545 ± 30	226.6
0.400	5	0		226.8 ± 0.7
0.400	18	0		253.8
0.393	1	0	442 ± 13	218.0 ± 1.9
0.393	1	0	778 ± 10	221.1 ± 1.1
0.385	3	0	1275	229.2
0.385	3	0	1148	228.8
0.385	3	0	683	225.2
0.385	3	0	590	224.2
0.385	3	0	402	212.5
0.385	3	0	256	203.8
0.400	1	20	490 ± 10	234.6 ± 0.5
0.325	1	20		220.2
0.325	3.2	20	465 ± 15	229.4
0.325	4	20		231.6
0.325	18.5	20	290 ± 10	260.1 ± 0.5
		Sample 5		
0.326	3	0		214.0 ± 0.2
0.326	5	0		224.4 ± 0.8
0.326	7	0	518 ± 52	224.2 ± 1.9
0.326	17	0		233.3 ± 1.6
0.408	3	0	833 ± 50	227.1 ± 1.0

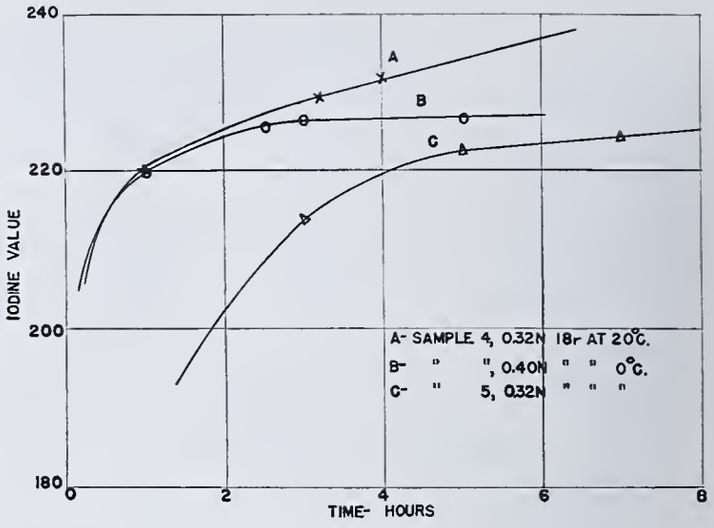

Figure 3. Iodine Values of Tung Oil with Woburn Solutions

In order to facilitate the interpretation of these results, the iodine values of samples 4 and 5 have been plotted against time of contact (Figure 3). The shape of these curves indicates the presence of two distinct halogen-consuming reactions proceeding at different rates.

Curve *A* shows that, with 0.32 *N* iodine bromide solution at 20° C., a rapid absorption of halogen takes place during the first hour, corresponding to an iodine value of about 220. This is followed by a much slower absorption corresponding to 3 or 4 units per hour.

In comparison, a 0.40 *N* solution at 0° C. (curve *B*) results in an equally fast absorption during the first hour and the value continues to rise slightly during the second and third hours but stays almost constant thereafter for several hours. Thus, the use of the more concentrated solution at lower temperature retards the secondary reaction without decreasing the speed of the primary absorption. However, even in this case, halogen absorption continues and a value of 253.8 is obtained after 18 hours' contact (Table VII, sample 4).

On the other hand, if the 0.32 *N* solution is applied at 0° C. (curve *C*), the initial reaction itself is retarded and the curve does not begin to flatten out until 6 to 7 hours after the addition of reagent.

The conclusion that two different reactions contribute to the halogen absorption is confirmed by the nature of the curve in Figure 4, obtained by plotting excess of reagent against iodine value. The steep branch of this curve, *D*, corresponds to conditions in which the primary reaction is incomplete. Above 590 per cent excess (point *A*) the slope of this curve abruptly decreases, *E*. From here on, the iodine value obtained depends to a much smaller degree on the excess of reagent present, indicating that the primary reaction is complete and the resulting value is determined by the extent to which the secondary reaction participates. Thus, under the conditions used, 590 per cent excess of reagent is just sufficient to complete the first, speedy absorption, without interference by the secondary reaction.

These observations recall the influence of time, temperature, and excess reagent on the Wijs iodine value of tung oil (*10*). In that case the two reactions involved are known to be a rapid addition of halogen to two of the three conjugated double bonds of the eleostearic acid radical and a slow subsequent addition to the third double bond. The true partial iodine value of tung oil was found to coincide with the intersection of the two branches of the curves obtained by plotting iodine value against either excess reagent or sample weight (*27*).

By analogy it appears, therefore, that the value of 224.2 obtained with 590 per cent on tung oil sample 4 is an intrinsic iodine value of this oil, whereas the other points on the curves are apparent iodine values.

In view of the observation by previous investigators, who used hydrogenation methods for determining the unsaturation of tung oil (Table I), the authors believe the conclusion justified that this value of 224.2 represents the true total unsaturation of the sample examined. None of the hydrogenation-iodine values for tung oil found in the literature are higher than 227. This fact, together with the present observation of a break in halogen absorption in the neighborhood of this value, indicates that the total unsaturation of tung oil is attained at this point. The higher values found with bromine vapor or upon prolonged exposure with iodine bromide solutions are then due to such secondary reactions as substitution, addition to secondary valences (*34*), or new double bonds formed during the determination (*31*, *40*).

This conclusion is corroborated by data obtained with beta-eleostearic acid shown below.

On the basis of these observations, we may define the conditions necessary for the determination of the total unsaturation of tung oil. Several procedures are possible, according to accuracy and speed required.

If greatest accuracy is needed, several values should be determined with 0.38 to 0.40 N reagent in an ice bath, using varying sample weights and the values plotted against excess of reagent as in Figure 4. The intersection of the two branches of this curve represents the total unsaturation.

As a quicker and more convenient method, the iodine value is determined as described in Method B, with a sample weight which will leave 650 to 800 per cent excess of halogen at the end of the reaction. Figure 4 shows that within these limits the dependence of the iodine value on excess is less than two units.

Finally, where speed is most important, a 0.32 N reagent may be employed for 1 hour at 20° C., using sample weights below 0.08 gram. This method will give the total unsaturation within 3 to 4 points, a margin of error which is no greater than that encountered in most of the bromine vapor addition methods.

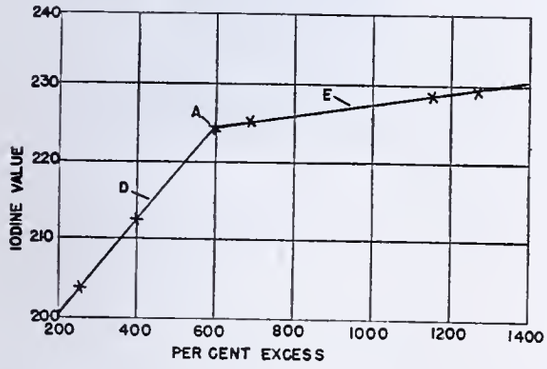

FIGURE 4. IODINE VALUES OF TUNG OIL WITH VARYING EXCESS OF WOBURN SOLUTIONS
3 hours at 0° C.

OTHER OILS AND FATTY ACIDS. Beta-eleostearic acid (melting point, 71.5° C) was prepared by repeated recrystallization of irradiated tung oil fatty acids in petroleum ether, using carbon dioxide to prevent oxidation. The average values found in several determinations are listed in Table VIII, together with the conditions used in each case. Similarly, values obtained on 9,11-linoleic acid (melting point, 53.4° C.) prepared according to Mangold (*26*) and on a blend containing 50 parts of this acid and 50 parts of soybean fatty acids, sample 2 (Table IX), are listed. A sample of raw oiticica oil, several years old, gave the value listed in Table VIII as the average of three determinations agreeing within ±0.8.

TABLE VIII. IODINE VALUES OF CONJUGATED COMPOUNDS

Sample	Normality of IBr Solution	Excess of Halogen	Temperature	Time	Iodine Values Found[a]	Iodine Values Calcd.
		%	° C.	Hours		
Beta-eleostearic acid	0.32	550–600	20	1	272.7	273.7
	0.40	600–2000	0	3	273.8	...
9,11-Linoleic acid	0.32	400–500	20	1	183.3	181.2
	0.32	400–500	0	3	182.6	181.2
	0.32	400–500	0	5	183.7	181.2
50% 9,11-acid + 50% soybean fatty acid	0.40	600–700	0	3	163.7	159.2
	0.32	400–500	20	1	160.6	159.2
Oiticica oil	0.40	800–1000	0	3	203.9	...

[a] Averages of 2 or 3 determinations each.

TABLE IX. WIJS AND WOBURN IODINE VALUES

Oil or Fatty Acid	Wijs Iodine Value	Woburn Iodine Value	Normality of Woburn Solution
Soybean oil, sample 1	130.5	126.5	0.36
Soybean oil, sample 2	131.8	127.2	0.36
Soybean pentaerythritol ester	127.6	128.4 ± 0.1	0.40
Soybean fatty acids, sample 1	137.8	136.0 ± 0.6	0.40
Soybean fatty acids, sample 2	138.8	139.6 ± 1.1	0.39
	138.8	137.2 ± 0	0.32
Linseed oil, sample 1	186.8	185.5 ± 0.1	0.36
Linseed oil, sample 2	185.0	185.8 ± 0.5	0.40
Linseed fatty acids, sample 1	194.2	196.4 ± 0.4	0.40
Linseed fatty acids, sample 2	190.3	191.1 ± 0.2	0.40
Linseed fatty acids, sample 3	191.2	186.9	0.36
Sardine oil, winterized	196.9	189.2	0.36
Sardine fatty acids, winterized	205.0	196.2	0.36
Teaseed oil	87.7	88.8 ± 1	0.32
Tall oil, distilled	169.5	203.8 ± 1.1	0.40
Perilla fatty acids	198.9	202.3 ± 0.2	0.40
Walnut oil	155.2	156.0 ± 0.1	0.32
Castor oil	87.4	88.9	0.32

This is significant, in view of the fact that this oil cannot be successfully dealt with by any of the known iodine procedures (*12*, *17*).

The fact that the proposed method is applicable not only to substances containing conjugated double bonds, but to nonconjugated fatty acids and oils as well, is demonstrated by the values for total unsaturation listed in Table IX. The Woburn values listed in this table were determined according to Method A, except that the concentration of the reagent varied between 0.32 and 0.40 N.

Except in the case of tall oil and sardine oil, these values agree with the Wijs iodine value within 1 to 5 units. The discrepancy with tall oil is understandable, in view of the presence of large proportions of rosin acids, sterols, and possibly other compounds, which react incompletely with Wijs reagent. The difference of approximately 8 units in the case of sardine oil, and the smaller differences with some of the other oils and fatty acids, are comparable to the discrepancies which result when other halogen-absorption methods are compared with each other.

The Woburn iodine values obtained on castor oil show that the hydroxyl group of ricinoleic acid is not attacked to any extent by 0.32 N iodine bromide solution.

Further data will be presented in another paper, in which the use of the Woburn method for the determination of the diene value and the composition of conjugated oils will be demonstrated.

Preparation of Woburn Iodine Solution

Woburn iodine solution consists of a 0.32 N iodine bromide solution in glacial acetic acid. The concentration should not vary more than ±0.01 N.

MATERIALS. Empyreuma-free 99.5 per cent acetic acid (bichromate test = 0.5 hours) (supplied by J. T. Baker Chemical Company as acid acetic, C. P. special, Lever Brothers), resub-

limed (reagent) iodine, C. P. bromine, 0.17 N sodium thiosulfate solution, 15 per cent C. P. potassium iodide solution and starch solution.

To prepare approximately 1 liter of the solution, dissolve 20.5 grams of iodine in 925 ml. of the acetic acid. This is best done in a 1-liter flask with the aid of occasional stirring and moderate heat (hot plate or steam bath). When all is dissolved, allow to cool to room temperature, measure out 25 ml. with a pipet, and titrate with 0.17 N sodium thiosulfate solution, using starch as indicator in the usual manner (*14*). From the titration, calculate the number of additional milliliters, Y, required to dilute the solution to 0.32 N, as follows:

a = ml. of thiosulfate solution used
b = grams of iodine equivalent to 1 ml. of thiosulfate solution

$$Y = 1773\,ab - 900$$

Then calculate the weight of bromine, X, necessary to double the halogen equivalent, as follows:

$$X = 22.7\,ab$$

Tare a graduated cylinder, containing about 25 ml. of acetic acid, on a scale accurate to 0.1 gram, and add X grams of bromine by means of an eye dropper. Add acetic acid until the volume in the cylinder equals Y ml. After the bromine solution in the cylinder is added to the remaining 900 ml. of iodine solution, the reagent is ready for use. If kept in the dark or in diffused daylight, it is stable for several months without substantial change in concentration.

Other Required Reagents

In preparing the 0.17 N sodium thiosulfate solution, follow the customary precautions (*14*), such as using freshly boiled distilled water (carbon dioxide–free and sterile) and filtering before use; 42.2 grams of C. P. sodium thiosulfate ($Na_2S_2O_3.5H_2O$) are required per liter of solution. Standardize this solution with resublimed iodine in the usual manner and calculate the iodine equivalent of 1 ml.

Prepare the 15 per cent potassium iodide solution and the starch indicator in the customary way (*14*).

Determination of Woburn Iodine Value

Method A. Conditions: 0.32 ± 0.01 N iodine bromide solution, 1 hour at 20° C.

Weigh accurately 0.06 to 0.16 gram of fatty acid or oil, depending on the expected iodine value (the excess of halogen should be from 500 to 800 per cent of the quantity absorbed), into a clean and dry glass-stoppered 250-ml. (8-ounce) bottle. Add 10 ml. of chloroform and swirl gently until sample is dissolved. Add exactly 25 ml. of 0.32 N iodine bromide solution, preferably from an automatic buret or pipet (Figure 5). Swirl and place the bottle in the dark inside a water bath kept at 20° C. After 1 hour add 20 ml. of 15 per cent potassium iodide solution, washing down any iodine bromide which may have collected in the neck or on the walls of the bottle. Mix and titrate with 0.17 N sodium thiosulfate solution in the ordinary manner, using starch indicator when the mixture has become pale yellow.

Blank determinations with 10 ml. of chloroform are made in the same manner.

The iodine value is calculated as follows:

$$I.\,V. = \frac{100\,(A - B)F}{S}$$

A = ml. of sodium thiosulfate solution used for blank
B = ml. of sodium thiosulfate solution used for sample
F = grams of iodine equivalent to 1 ml. of sodium thiosulfate solution
S = weight of sample in grams

Method B. Conditions: 0.40 ± 0.01 N iodine bromide solution, 3 hours at 0° C.

This method is recommended for tung and oiticica oils. Ice is used to cool the reaction mixture and thin-walled Erlenmeyer flasks are required to assure proper cooling.

Weigh out 0.060 to 0.075 gram of oil in a thin-walled glass-stoppered Erlenmeyer flask, add 10 ml. of chloroform to dissolve the oil, and swirl the flask in a beaker containing crushed ice for 3 to 5 minutes. Continue swirling and deliver 25 ml. of 0.40 N iodine bromide solution. Note time of the addition of reagent. Wet the glass stopper with potassium iodide solution to prevent evaporation of the halogens, but do not allow any of this solution to come in contact with the reaction mixture. Continue to swirl in the ice bath for 2 to 3 more minutes, then immediately place the flask in a container almost filled with crushed ice in the dark, and allow it to remain there for 3 hours after the addition of reagent. After this period proceed as in Method A, but keeping the concentration of sodium thiosulfate preferably between 0.21 and 0.22 N.

Note. With some compounds, such as 9,11-linoleic acid, best results were obtained by following the details of Method B, but using 0.32 N rather than 0.40 N iodine bromide solution.

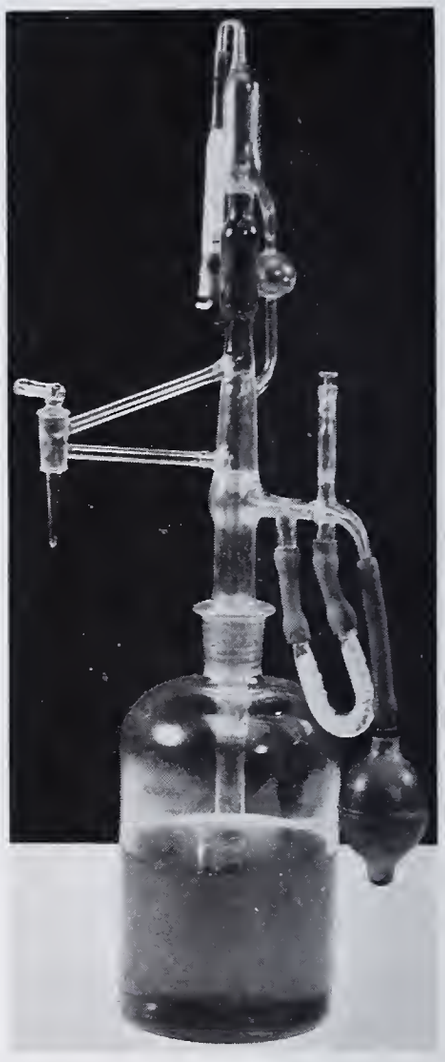

Figure 5. Automatic Pipet with Stock Bottle
Made by Scientific Glass Apparatus Co.

Acknowledgment

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Hydrofluosilicic Acid Method for Determination of Quartz

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HYDROFLUOSILICIC acid affords a differentiation between quartz and other silicon-containing compounds such as the silicates. This reagent dissolves most silicates very rapidly, while its action on quartz is exceedingly slow. However, in spite of many attractive features, its use as a reagent for estimating the amount of quartz in siliceous materials (*2*, *5*) presents certain difficulties, especially when the method is extended to finely divided granular materials such as rock dusts. Factors such as temperature, the size distribution of the particles, and the magnitude of the control blank on the reagent, plus certain manipulative difficulties in carrying out the analysis, must be taken into account. Investigation of some of these factors in these laboratories has cleared up some of the difficulties and extended the method to the analysis of settled dusts.

"Settled dust" is collected and examined as a routine procedure in estimating atmospheric contamination due to dustiness in the industrial environment. As the name implies, it is the dust which has settled on rafters, window ledges, roofs of buildings, and various other sites, relating to the industrial environment.

A line must be drawn somewhat in defining certain material as settled dust, which certainly does not include pebbles, match sticks, bits of rubbish, etc. It has been the practice of this laboratory, therefore, to sieve all samples through a 200-mesh sieve and to analyze this sieved material as "settled dust". The average diameter of such granular material is usually about 6 to 8 microns. However, if samples ground to pass a 200-mesh sieve are again sieved through a 325-mesh sieve, the average diameter of the particles retained on the 325-mesh sieve may vary from 48 to 90 microns (*3*).

According to the hydrofluosilicic acid procedure as described by Knopf, the loss of quartz from a pure quartz sample ground to pass a 150-mesh screen is 1.4 per cent per 48 hours after 14 days of treatment. The rate of decomposition was found to differ considerably in different silicates. Certain refractory silicates required a week or more to decompose (*5*).

Moke (*6*) in a study of foundry dusts (*4*) used Knopf's hydrofluosilicic acid procedure and determined the solubility of quartz on particles of 10 microns and less. The loss was from 13.6 to 48.5 per cent in 48 hours. In this connection Moke says that if sufficient time and acid were employed, all the quartz would undoubtedly go into solution. The rate of solubility increases greatly with decrease in grain size.

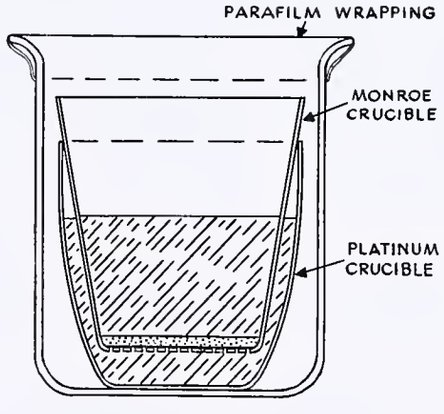

Figure 1

Care must be exercised (*5*) not to raise the temperature during the hydrofluosilicic acid treatment, because hydrofluosilicic acid (H_2SiF_6) decomposes on heating into silicon tetrafluoride (SiF_4) and hydrofluoric acid (HF) which will readily attack free silica.

Material and Reagents

Samples of settled dust were employed in this investigation. The quartz sample used as a control was Brazilian quartz, sieved through a 200-mesh screen.

Three grams of silica gel were added to 500 grams of a commercial grade of hydrofluosilicic acid.

A 1 to 1 nitric acid solution was used.

Procedure

A sample of dust weighing 0.2 to 0.5 gram is taken for analysis. It is ignited in a platinum crucible and an aliquot of the residue is transferred to a Munroe crucible. (This is similar to a Gooch crucible but is of platinum and contains a platinum mat. Such crucibles may be obtained through dealers in platinum laboratory ware.) The Munroe crucible is set inside an ordinary platinum crucible. Ten milliliters of hydrofluosilicic acid reagent are added to the sample in the Munroe crucible and the crucibles are set inside a 100-ml. beaker (Figure 1), which is covered with Parafilm (a gas-tight material obtained from the Fisher Scientific Company), placed in the electric refrigerator at 10° C., and allowed to remain there for 48 hours.

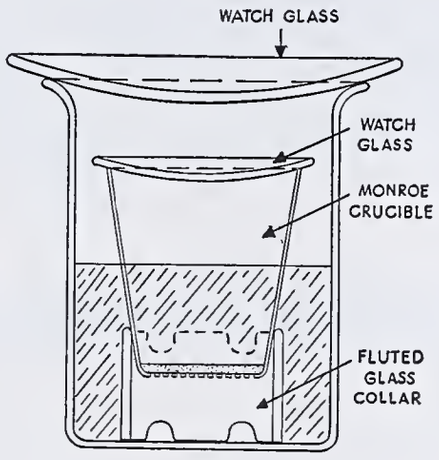

FIGURE 2

The Munroe crucible is now transferred to the filter funnel and the reagent is drawn off by suction. The filter flask used for this purpose is partly filled with water to prevent etching. The filter is washed with cold water, the filter flask is emptied, and the filter is finally washed with nitric acid. The Munroe crucible is now set in a 150-ml. beaker on a glass collar which is fluted at both ends (Figure 2). The crucible is covered with a small watch glass, nitric acid is added, and the beaker is covered over with a watch glass. The contents are warmed at about 70° C. for 0.5 hour. The nitric acid is filtered off by suction, the filter is washed with water, and the crucible and contents are dried at 120° for one hour, cooled, and weighed.

The hydrofluosilicic acid and the nitric acid treatment is now repeated until a constant loss in weight is obtained. At this point an aliquot of residue is transferred to a platinum crucible and ignited to constant weight in the muffle furnace.

The residue may be examined petrographically at any time during the course of these treatments, and finally total silica may be determined on it by hydrofluoric acid.

Table I gives a typical result obtained by this method when applied to siliceous settled dusts.

Table II gives the results obtained when quartz is treated with hydrofluosilicic acid with and without the addition of silica gel.

TABLE I. DETERMINATION OF QUARTZ

(Settled Dust 686, sample 0.3143 gram, 6 treatments with H_2SiF_6)

Treatment	Loss in weight *Gram*
1	0.0863
2	0.0216
3	0.0115
4	0.0082
5	0.0061
6	0.0045
Total	0.1382

0.1665 gram of residue treated with H_2F_2 gave a loss of 0.1588 gram. Quartz (uncorrected), 44%

TABLE II. DETERMINATION OF QUARTZ

Sample 0.2380 gram, 4 treatments with H_2SiF_6 (SiO_2 added)

Treatment *Hours*	Loss in Weight *Gram*
48	0.0057
72	0.0033
24	0.0008
24	0.0016

Average loss for 48 hours, 1.4%

Sample 0.2140 gram, 2 treatments with H_2SiF_6 (no SiO_2 added)

48	0.0120
48	0.0182

Average loss for 48 hours, 7.1%

Sample 0.1789 gram, 5 treatments with H_2SiF_6 (SiO_2 added)

48	0.0004
48	0.0003
48	0.0008
48	0.0002
48	0.0003
Total	0.0020

Loss after ignition, 0.0024. Blank on empty platinum crucible. Loss, 0.5 mg. or 0.1 mg. per 48 hours. Loss for quartz (corrected), 0.38 mg. per 48 hours or 2.1% per 48 hours.

Discussion of Method

The solution of quartz by the reagent is greatly reduced by employing a low constant temperature (10° instead of room temperature). This temperature is purely arbitrary. The electric refrigerator was used because of its availability, convenience, and the relative constancy of the temperature compared to that which obtains in the laboratory. The procedure described here can be employed with advantage even at room temperatures, if the sample is not too finely divided. However, the magnitude of the quartz blank in the case of a dust (13.6 to 48.5 per cent in 48 hours) is so great as to preclude the use of the method at ordinary room temperatures.

The addition of silica gel to the hydrofluosilicic acid makes for a low constant blank, probably by removing any excess hydrofluoric acid which may be present. Quartz control samples weighing 0.2 gram lost from 6 to 9 per cent by weight when untreated hydrofluosilicic acid was used. With the treated hydrofluosilicic acid the quartz samples lost only 0.4 mg. in 48 hours. This compares with 2.8 mg. in 48 hours found by Knopf using 150-mesh quartz (*5*). Different lots of hydrofluosilicic acid gave concordant results.

The Munroe crucible must be tested for cracks before each complete analysis. It should be able to retain freshly precipitated calcium oxalate. Cracks are easily mended (*1*).

The use of nitric instead of hydrochloric acid prevents the formation of cracks and solution of the platinum. Controls with nitric acid showed losses of only 0.1 mg. per 48 hours in the course of a complete analysis; 48-hour treatments were found most convenient and almost as efficient as 24-hour treatments and allow the analysis to be carried on over week ends.

The initial ignition of the sample is to be avoided in certain cases—for example, in limestones containing small amounts of quartz, when a preliminary hydrochloric acid treatment is to be preferred. The use of the Munroe crucible avoids the repeated transfer of sample onto a filter paper and therefore avoids the errors inherent in such operations. The time required for manipulative detail is cut down to about 30 minutes for each 48-hour period; this enables samples to be removed and started again within the same day.

Summary

A method for the analysis of the quartz content of granular material using hydrofluosilicic acid has been extended to include "settled dusts". Mechanical losses and losses due to the solution of quartz by the reagent have been greatly reduced. There is a substantial saving of time in an analysis which may take as long as 2 weeks to complete.

Acknowledgment

The author wishes to thank R. W. Schayer for his assistance in carrying out most of the experimental detail.

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Determination of Traces of Tin in Malt Beverages

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THE presence of traces of tin in beer, due to solution from tin surfaces in brewery and dispensing equipment, has long been known to produce haziness and turbidity in the finished beverage. The relatively high intensity of the colloidal haze produced seems out of all proportion to the minute quantities of tin involved. As little as 0.1 part per million will affect the clarity of the beverage.

Goob (*3*) in 1912, writing on tin turbidities, noted the unsatisfactory state of the quantitative methods for the chemical determination of traces of this metal. A search of the literature failed to reveal any specific methods or improvements of methods for the chemical determination of these traces of tin in beer since that time. There are many published chemical methods for determining small amounts of tin in food and related products, but these, without exception, are for quantities greatly in excess of those which may cause trouble in beer. Some of these methods might be adapted to determining traces of tin by using extremely large volumes of sample, but this is not a completely satisfactory expedient—for instance, it would be necessary to examine 4 liters of a beer containing 0.25 part per million of tin in order to recover 1 mg. of tin. Large charges necessitate increased volumes of reagents and solutions and the effective increase in sensitivity thus obtained is not great. In most cases, the minute traces of tin involved would be lost in the elaborate separations used.

The development of spectrographic analysis (*4*) has brought a new tool which may be applied to this problem, but this method requires special training and expensive apparatus and is beyond the means of the average analyst.

Nearly all the methods in the literature for determining tin in biological materials utilize a tedious wet digestion for destroying the organic matter prior to separating and measuring the tin and avoid the more convenient ashing. During ashing, the tin changes to tin oxide, which is extremely refractory and resists solution in all common acids. The widespread use of the wet digestion has probably been due to a desire to avoid the formation of this refractory tin oxide.

Tin oxide readily becomes soluble when fused with a number of different substances. A method for determining tin in foods, which utilizes an ashing and a fusion of the ash, was described (*2*) in 1928. This method is not sufficiently sensitive for determining tin in beer, but the treatment of the sample prior to actual analysis is of interest.

Until recently, there has been a notable lack of good specific sensitive organic reagents for detecting tin. The older tests utilized, in one way or another, the reducing properties of the stannous ion or the oxidizing properties of the stannic ion. In 1936, Clark (*1*) recommended the dimercaptobenzenes as sensitive and specific reagents for tin. Tin combines with these organic reagents to give red precipitates.

By combining the ashing and fusion technique for the preliminary preparation of the sample with the new organic reagent, it was possible to develop a simple, rapid, and accurate method for the determination of traces of tin in beer. This quantitative determination also serves as a qualitative test, thus ensuring that the measured factor is actually tin. The availability of the reagent (1-methyl-3,4-dimercaptobenzene, obtainable from the Organic Products Company, 17 Thompson St., New York, N. Y., or the British Drug House through the Eastman Kodak Company, Rochester, N. Y.) and the perfection of a stable solution add further to its convenience.

Reagents

Fusion Mixture. Quickly grind (to avoid local evolution of hydrocyanic acid by atmospheric carbon dioxide) with a mortar and pestle 12.5 grams of sodium cyanide with 37.5 grams of anhydrous sodium carbonate to give a homogeneous powder.

Hydrochloric Acid. Dilute 1 volume of concentrated hydrochloric acid with 1 volume of water.

1-Methyl-3,4-dimercaptobenzene (Dithiol) Solution. Warm the dithiol slightly so that it liquefies, dissolve 0.25 ml. in 10 ml. of thioglycolic acid, and dilute to 200 ml. with 95 per cent ethyl alcohol. Store in full tightly corked small bottles in the dark. The reagent is stable if protected from air, but in a bottle that has been repeatedly opened will frequently become oxidized and no longer give the reaction with tin. Thus, the reagent from an opened bottle should be tested against a known tin solution before adding it to the sample. (The reagent has a strong sulfidic odor and may be deodorized, if spilled, by washing with a dilute iodine solution.)

Tin Standard. Dissolve 1.90 grams of stannous chloride dihydrate in 20 ml. of the hydrochloric acid solution and dilute to 1 liter with water. This solution should be prepared fresh, as it is not very stable.

Gum Arabic Solution. Dissolve 100 grams of powdered gum arabic (U. S. P.) in 1 liter of hot water containing 100 ml. of 0.1 per cent phenyl mercuric acetate solution. When dissolved, filter through paper in a hot water funnel. This solution is stable and will not get moldy.

Method

Char and then ignite (preferably in a muffle at about 550° C.) 100 to 200 ml. of well-mixed, degassed beer in a silica dish to give a white fluffy ash. Avoid too high a temperature during ashing to prevent fusion of the ash. Transfer the ash by brushing to a No. 00 Coors high-form porcelain crucible, tamp down ash in the crucible, and cover with 1 gram of the fusion mixture. Fuse over a Méker burner for about 15 seconds, holding the crucible with a pair of tongs and swirling, so that the melt is given

Table I. Recovery of Added Tin

Sample Taken for Analysis	Tin Added		Tin Found	
Ml.	*Mg. per charge*	*P. p. m.*	*Mg.*	*P. p. m.*
200	Nil	Nil	Nil	Nil
200	0.05	0.25	0.05	0.25
200	0.03	0.15	0.03	0.15
200	0.03	0.15	0.04	0.20
100	0.04	0.40	0.04	0.40
100	0.075	0.75	0.075	0.75
100	Nil	Nil	Nil	Nil
100	0.05	0.5	0.05	0.5
100	0.05	0.5	0.04	0.4
100	Nil	Nil	Nil	Nil
100	0.01	0.1	Nil	Nil
200	0.02	0.1	0.01	0.05
100	0.015	0.15	Nil	Nil
200	0.03	0.15	0.015	0.08
100	0.025	0.25	0.025	0.25
100	0.05	0.5	0.04	0.4
100	0.05	0.5	0.04	0.4

a rotating motion. Do not overheat or fuse too long. Cool and place the crucible upright in a 50-ml. Pyrex beaker which is covered with a watch glass. All further operations, because of the poisonous character of the liberated hydrocyanic acid gas, must be conducted in a well-ventilated hood.

Put 5 ml. of the hydrochloric acid solution directly into the crucible by means of a pipet introduced through the space between the watch glass and the beaker spout. Allow it to react until the evolution of gas ceases. Remove the watch glass and wash down its undersurface with water, permitting this wash water to run into the crucible. With a thin stirring rod overturn the crucible and heat. This will generally cause further evolution of gas from the crucible. Continue heating and stirring until no further evolution of gas takes place, pick up the crucible by means of the stirring rod, and wash with water. Cover the beaker with the original watch glass and boil down the combined solution and washings to a volume of less than 10 ml. Transfer and wash this solution into a 15-ml. centrifuge tube graduated at 10 ml., make up volume to 10 ml., mix, and centrifuge at high speed. Transfer the clear supernatant liquid to a test tube, add 0.5 ml. of the tin reagent, mix, and heat for 1 minute in a slowly boiling water bath. Cool, add 2 ml. of the gum arabic solution, cork, and shake thoroughly. Compare the resulting colored turbidity with standards by reflection, using daylight.

Preparation of Standards. Dilute 20 ml. of fresh tin standard solution and 10 ml. of hydrochloric acid to 200 ml. with water. Pipet the following suggested quantities of this standard solution into a series of test tubes: 0.0, 0.1, 0.25, 0.50, 0.75, and 1.0 ml. (equivalent to 0.0, 0.01, 0.025, 0.05, 0.075, and 0.1 mg. of tin, respectively). Add 1 ml. of hydrochloric acid solution and make volume up to 10 ml. with water. Add 0.5 ml. of tin reagent, mix, and heat in a slowly boiling water bath for 1 minute. Cool, add 2 ml. of gum arabic solution, cork, and shake thoroughly. Standards thus prepared are stable for a month or more if kept corked and in the dark. After standing, they should be vigorously shaken before using.

Recovery of Added Tin

Since there is no other reliable method available against which the accuracy of the method could be checked, recourse was had to a determination of added tin. Beers that were brilliant on chilling are found to give a negative test for tin by this method. Such beers were assumed to be tin-free and tin was added in varying amounts. After standing some time, they were analyzed and the results are given in Table I.

Interferences

As shown by Clark, numerous heavy metals give varicolored precipitates with this reagent, but the red color of tin is characteristic; the only metal which approaches this color is bismuth, which exhibits a completely different shade of red. Other metals, if present to excess, can interfere in the method by masking the color of the tin precipitate. Iron, the only metal that is liable to be encountered in excess in beers, will not interfere in the test because during the cyanide fusion it is converted to compounds which, on later acidification, form the insoluble Prussian blue. The Prussian blue is then removed along with the other insoluble salts.

Table II shows the effect of the presence of 10 parts per million of interfering heavy metals on the determination of tin in a beer containing 0.5 part per million of tin.

Except for iron, the results in Table II are of no practical interest. Excess iron is removed in the course of the analysis and the copper content of beer is effectively limited by the normal removal of excess copper by the yeast. None of the other metals is present in beer in quantities sufficient to cause interference.

In the case of a rare sample in which large amounts of the interfering metals may be present, they may be dissolved out of the ash by dilute acids, leaving the insoluble tin oxide behind. This purified precipitate containing the tin oxide, after filtration, washing, and re-ashing, can then be treated as in the regular method. This procedure tends to reduce the sensitivity of the method and great care is needed to prevent the fine tin oxide precipitate from mechanically passing into the acid-soluble filtrate. In this laboratory, where many different samples representing all types of beers and ales have been examined, as yet no sample has been encountered containing a sufficient quantity of interfering metal to necessitate the use of this longer and less sensitive modified procedure.

This method has been tried on a number of different types of materials including several foods, water, and other beverages and the indications are that it may readily be adapted to materials other than beer. It is especially useful where only small amounts of sample are available.

Table II. Effect of Presence of Heavy Metals on Determination of Tin

(Beer containing 0.5 p. p. m. of tin)

Metal Added (10 P. P. M.)	Results
None	0.5 p. p. m. tin, red turbidity
Bismuth	No tin, yellow precipitate
Cobalt	No tin, dirty yellow precipitate
Copper	No tin, black precipitate
Iron	0.5 p. p. m. tin, no interference
Lead	0.5 p. p. m. tin, no interference
Manganese	0.4 p. p. m. tin, orange turbidity
Nickel	0.1 p. p. m. tin
Zinc	0.25 p. p. m. tin, orange turbidity

Summary

Minute traces of tin, much below the practical limit of sensitivity of existing chemical analytical methods, produce haziness in beers and ales. A method is described for determining such traces of tin, wherein the beer is ashed and the ash fused with a sodium carbonate–sodium cyanide mixture. After solution in acid, the tin is determined by the intensity of the color of the red precipitate produced by a solution of the specific organic reagent 1-methyl-3,4-dimercaptobenzene (dithiol). Recoveries and interferences are noted. This method may be adapted to the determination of traces of tin in other foodstuffs, water, and biological materials.

Acknowledgment

The writer wishes to thank Roy W. Seaholm for conducting many of the determinations reported here.

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Rapid Photometric Determination of Ascorbic Acid in Plant Materials

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THE method described here is an adaptation and modification of the photometric determination of ascorbic acid in blood serum as reported by Mindlin and Butler (*7*) and modified by Bessey (*1*) to include colored or turbid solutions and plant tissue extracts. The new features reported here involve chiefly the methods of extraction, filtering instead of centrifuging, and various changes in concentration of reagents to permit measurement of a wider range—1 to 14 micrograms—of ascorbic acid in the final aliquots. These modifications were developed especially to permit high-speed work on large numbers of plant samples daily, as in evaluating thousands of individuals among segregating populations incidental to breeding for high vitamin C content in vegetables. By these modifications, an analyst with two assistants can readily run 120 samples daily.

Since the photometric method has been discussed by others (*1*, *7*) and the use of the indophenol dye has been reviewed by King (*5*), the principles and the advantages of the basic method need not be reviewed here.

Apparatus and Reagents

The fresh plant tissues were first reduced to a fine pulp in the Waring blender as described by Davis (*3*), using two containers alternately to increase the output of the machine.

The following solutions were required: (1) 3 per cent metaphosphoric acid; (2) sodium citrate buffer of 211 grams of citric acid in 2 liters of 1 *N* sodium hydroxide; (3) a buffer at pH 3.6 (±0.1); mixture of 3200 ml. of solution 1 and 868 ml. of solution 2; and (4) a solution of 2,6-dichlorophenolindophenol containing 34.4 mg. (Eastman Kodak Co. preparation) in 1 liter of water. Solutions were used within 5 days and always stored in the ice chest overnight. Control of pH was accomplished by means of the McGinnes (*6*) glass electrode. The purity of the ascorbic acid used for preparing the calibration curve, and for the recovery experiments, was tested by titration with standard iodine solution as described by Bessey and King (*2*). Quantitative titration values were obtained within 2 per cent of theory.

The photoelectric colorimeter described by Evelyn (*4*) was used with green filter No. 520 (transmission limits 495 to 550 millimicrons). Six dozen 17.5 × 2.2 cm. (7 × 0.875 inch) absorption test tubes were selected which agreed to within 0.25 galvanometer unit.

Procedure for Calibrating Standard Curve

A fresh solution of ascorbic acid is prepared by dissolving 25 mg. in 250 ml. of buffer at pH 3.6 (solution No. 3). (After 2 days, at 4° C., this solution lost 5 per cent of its reducing power.) One- to 14-ml. amounts, by 1-ml. increments, are added to 100-ml. volumetric flasks, which are then made to volume with the buffer at pH 3.6. Five-milliliter portions of the dye solution are added to each of a series of colorimeter test tubes with a pipet reserved for this purpose, thus assuring as near the same quantity of dye as practicable in all experiments.

The colorimeter is set at 100 per cent transmission, using a tube containing 5 ml. of buffer (pH 3.6), 5 ml. of the dye, and a few crystals of ascorbic acid for complete decoloration; this is conducted in triplicate to assure a correct galvanometer setting. The center setting (without any tube in the instrument) is then recorded and maintained constant for the subsequent determinations: five milliliters of a sample solution are quickly delivered from an Ostwald pipet into 5 ml. of the dye and shaken vigorously about 5 seconds; a reading is taken 15 seconds after initial mixing and again at 30 seconds. After observing the values for all 14 samples, a blank reading in triplicate is obtained using 5 ml. of the buffer. The differences between the 15- and 30-second readings did not exceed 0.5 galvanometer unit and the average of the two was used.

Table I. Calibration Data for Photometric Determination of Ascorbic Acid[a]

Micrograms/Ml.[b]	Galvanometer Readings[c]	Photometric Density ($2 - \log_{10} G$)[d]	Log *G* Sample − Log G Blank: Observed	Log *G* Sample − Log G Blank: Calculated[e]	Ratio[f] (Observed × 100 / Calcd.)
0 (blank)	20.50	0.6830	....	....	...
1.006	23.63	0.6220	0.0610	0.0581	105.0
2.012	26.13	0.5750	0.1080	0.1024	105.5
3.018	28.13	0.5435	0.1395	0.1467	95.0
4.024	31.63	0.4930	0.1900	0.1912	99.4
5.030	35.63	0.4425	0.2405	0.2355	102.1
6.036	39.00	0.4060	0.2770	0.2799	99.0
7.042	42.63	0.3680	0.3150	0.3243	97.2
8.048	47.38	0.3220	0.3610	0.3686	98.0
9.054	54.00	0.2656	0.4174	0.4130	96.1
10.060	60.50	0.2182	0.4648	0.4573	101.6
11.066	66.00	0.1805	0.5025	0.5017	100.3
12.072	74.25	0.1293	0.5537	0.5461	101.3
13.078	81.25	0.0901	0.5929	0.5904	100.3
14.084	87.75	0.0568	0.6262	0.6348	98.7

[a] 5 ml. of dye (34.44 mg. of 2,6-dichlorophenolindophenol per liter) mixed with 5 ml. of sample solutions containing 1 to 14 micrograms of ascorbic acid per ml.
[b] Ascorbic acid stock solution contained 25.18 mg. dissolved in 250 ml.
[c] Galvanometer was read to nearest quarter division and mean of 15- and 30-second readings used.
[d] Corrected for slight deviations from true linearity of relation between current and galvanometer deflection.
[e] Calculated from best line (derived by method of least squares) satisfying observed points.
[f] Coefficient of variation = 1.1%.

In Table I the data obtained for the calibration are presented. By the method of least squares, the equation for the line best satisfying the experimental points was found to be: $Y = 0.0441\ X + 0.0137$, where X = micrograms of ascorbic acid per ml. and Y = the log of the galvanometer readings for samples minus that for the blank (Figure 1). The coefficient of variation for the deviations from the line was only 1.1 per cent. A repetition of the calibration curve after a period of 5 weeks resulted in a similar series of points varying by only about 1 per cent from the original calibration.

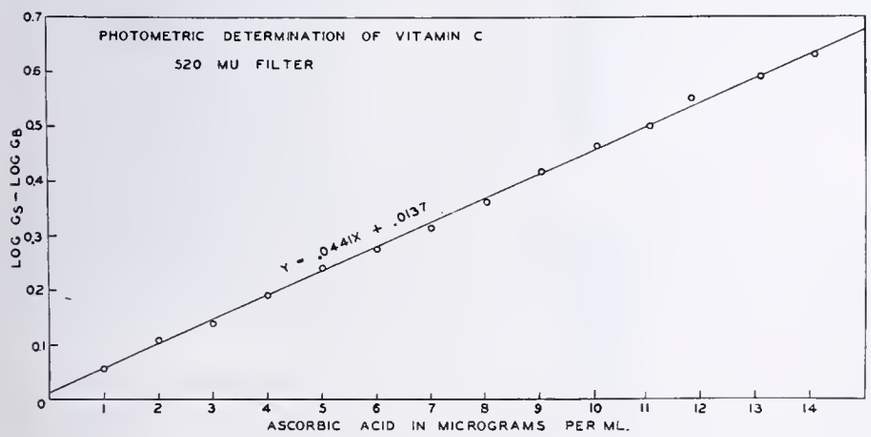

FIGURE 1

Procedure for Plant Materials

Application to Beans and Cabbages. From a dispensing buret 100 ml. of 3 per cent metaphosphoric acid are added to the container of the blender; 25 grams of fresh tissue, weighed to the nearest 0.1 gram, are added and mixed at high speed for 2 minutes. The fresh vegetable tissues are thus reduced to an extremely fine pulpy suspension, and a temperature rise of about 5° C. occurs. About half of the contents is then filtered through a dry fluted No. 12 Whatman filter paper. Since the filtrate comes through cloudy at the start, about 10 ml. are discarded. About 10 ml. of clear filtrate are collected in a dry Erlenmeyer flask.

The aliquot of filtrate taken for dilution will depend upon the approximate vitamin content of the sample. A volume is selected which is expected to contain between 200 and 600 micrograms, so that after dilution to 50 ml. the final concentration in the aliquots analyzed may conveniently lie between 4 and 12 micrograms per ml. For cabbage, which may contain about 40 to 90 mg. of ascorbic acid per 100 grams of fresh tissue, a 3-ml. aliquot is adequate, whereas for snap beans, containing about 20 to 40 mg. per 100 grams, a 5-ml. aliquot is taken.

Aliquots of the clear filtrates are transferred to 50-ml. volumetric flasks and a sufficient quantity of sodium citrate buffer (solution No. 2) is added to bring the pH to approximately 3.6; in the case of cabbages, beans, and several other vegetables studied, 0.25 ml. of buffer per ml. of aliquot was required. The mixture is then made to volume with the citrate-phosphate buffer (solution No. 3). The final pH should be 3.6 ± 0.1. When many samples of similar material are analyzed in groups of 24, a pH determination on occasional samples will permit adequate control.

In calculating the ascorbic acid content of plant tissue the water in the sample must be taken into account. In the present investigation the results obtained with the recovery experiments indicated that the vitamin is distributed in the liquid phase of the mix, with no measurable amount absorbed.

As a test of the accuracy of the method, the recovery of ascorbic acid was studied. Since a great deal of air is whipped into the extracting solution during the 2-minute stirring period, the possibility existed that oxidation might necessitate the use of an empirical correction factor. This was shown unnecessary, however, by quantitative recoveries when buffered solutions of ascorbic acid, at various concentrations, were carried through all the steps in the analysis. The possibility that ascorbic acid oxidase might partially destroy the vitamin during the analysis was also excluded by the quantitative recoveries obtained. Recovery experiments are complicated by the difficulty in obtaining uniform 25-gram samples of plant tissue. Four separate series, using individual cabbages, were conducted in the following manner:

The head was sliced along its polar axis and 25-gram sections were cut for analysis; five alternate sections were supplemented with different amounts of pure ascorbic acid and the average value for the unsupplemented five sections was used in the recovery calculations. A fresh solution containing 1 mg. of ascorbic acid per ml. was added—4, 6, 8, 10, and 12 ml., respectively—to each section before conducting the extraction.

The average recovery for the 20 separate analyses was slightly high, 103 ± 1.3 per cent. However, in these experiments a difference in total vitamin concentration, between supplemented and unsupplemented extracts, is being measured.

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Contribution 11 of the U. S. Regional Vegetable Breeding Laboratory, Bureau of Plant Industry, U. S. Department of Agriculture, Charleston, S. C.

A Constant Mercury Level for the Dropping Mercury Electrode

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FROM the Ilkovic equation for the diffusion current and Poiseuille's equation for the flow of liquids through capillaries, it can be shown that the diffusion current is proportional to the square root of the pressure or to the square root of the mercury height—i. e., $i_d = k\sqrt{h}$—for constant concentration and temperature. In a single analysis, the amount of mercury used is small; thus if a large reservoir is used, the height is essentially unchanged during the analysis, but it becomes inconvenient to correct the height manually if a series of analyses is being run.

Mueller (*1*) described a simple device for maintaining a constant mercury pressure, using the principle of the Mariotte flask. In these laboratories a floating bulb valve device, described below, has been used satisfactorily for over a year.

A ball, *B*, attached to the centering tube floats on the surface of the mercury pool which is to be kept at constant level. The hemispherical ground end of the capillary, *C*, rests on a ground projection blown on the upper side of *B*. Capillary *C* is joined to the reservoir, *A*, which sits loosely on the lower one. There is a hole on the upper side of *B* for introducing mercury for ballast. If sufficient care is taken in grinding *C* to fit *B*, the lower level is kept constant to within 1 mm., which is sufficiently constant for polarographic purposes.

The lower container may be connected to the dropping capillary by means of rubber tubing. If there is danger of clogging the capillary by contamination from the rubber, the two concentric movable tubes, *D*, may be used. The head necessary to obtain the proper drop time can easily be adjusted and the mercury is in contact with only the very small area of rubber resulting from the seal. The electrical connection, *E*, was made by sealing into the Pyrex tube a thin-walled piece of platinum tubing closed at both ends.

In order to stop the flow of mercury, the working cell is replaced by vessel *F*, which is tightly fitted to the rubber stopper on the capillary tube. The outer jacket of *F* may be filled with a saturated salt to protect the agar bridge if an external reference electrode is used.

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Determination of Grease in Sewage, Sludge, and Industrial Wastes

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Prevailing methods for determining the grease content of sewages, sludges, and liquid industrial wastes permit many errors. Recognizing the need for a standard procedure which will give a reliable measure of the true content of "grease" the authors have devised a procedure which eliminates or greatly reduces most of the sources of error. Application to a variety of samples gave results with differences from the means averaging 2.0 per cent. Results of analyses are much less influenced by variations of technique and of solvent than is the case with older methods.

IT HAS long been recognized that certain errors are present in prevailing methods for determining grease in sewage and sludges. Because of the need for more accurate procedure, W. T. Knowlton, City of Los Angeles Bureau of Engineering, called together a committee to study the problem. This group consisted of Mr. Knowlton: V. W. Thews and A. A. Appel, Maintenance Division, Sewer Department of the City of Los Angeles; T. C. Wilson, Los Angeles City Bureau of Standards; and the authors, who conducted the research. The other members of the committee assisted by suggestions and by making many analyses. More than ordinary expressions of appreciation are due these men for their part in this work.

In considering the accuracy of methods for determination of grease, it is necessary to have an understanding of what is sought. From the viewpoint of the sanitary engineer, a grease analysis should include those materials which impart greasy characteristics to the waste when they are present in sufficient quantity. It is also desirable to include oil, since oil differs from grease only in melting point. The material separated from sewage in grease analyses always contains both solid and liquid substances. If the solid constituents are present in sufficient quantity, the mixture is solid at room temperature and may properly be called grease, but if the liquids predominate the mixture remains liquid and should be called oil. In this discussion the word "grease" may be understood as meaning "grease or oil".

Sources of Errors in Existing Methods

Prevailing methods for determining grease in sewage, sludges, and many other fluid materials essentially involve acidifying the sample, evaporating to dryness, extracting thoroughly with a suitable solvent, and evaporating the grease-laden solvent so that the grease may be weighed. Consideration of such methods shows that the following errors may be serious.

VOLATILIZATION OF GREASE WHEN SAMPLE IS DRIED. When samples are evaporated to dryness, a part of the grease is lost along with the steam. The possible magnitude of this error was demonstrated experimentally for several greases.

The greases were prepared for use by holding in a stoppered bottle in a melted condition for at least 24 hours, after which clarified grease was pipetted out, except in the cases of raw sludge and digested sludge greases, which were recovered from analyses.

Samples were weighed into tared conical flasks and distilled water was added. The flasks were placed on a hot plate, and as the water evaporated more was added until 500 ml. had been used. The grease was then dried and weighed.

Table I shows that the losses were variable, reaching 25 per cent for grease from sewage skimmings. It is not possible from these results to predict the exact magnitude of the losses in analyses, but it is evident that they are likely to be an important source of error.

REVERSION OF FATTY ACIDS TO INSOLUBLE SOAPS. Insoluble soaps are an important factor in the greasiness of sewage; hence they should be included in grease analyses. Since they can be dissolved only with difficulty, it is common practice to acidify the sample and thus liberate the fatty acids which constitute about 95 per cent of the soap.

When hydrochloric acid is used for this purpose, the reaction may be represented by the equation

$$Ca(RCOO)_2 + 2HCl \rightarrow CaCl_2 + 2\ RCOOH$$

If the sample is dried, the above reaction reverses, regenerating the insoluble soaps. To prove that the reverse action can occur, 117 mg. of fatty acids from sewer grease and 144 mg. of calcium chloride dihydrate were mixed with ether and water in a dish, and evaporated to dryness. Thirty-four per cent of the fatty acids were converted to insoluble soaps. More intimate contact and longer drying time, such as might prevail in a sludge analysis, might easily result in conversion of most of the fatty acids into insoluble soaps.

Theoretically, the difficulty can be overcome by using sulfuric acid, but one is faced with the task of adding exactly enough to convert all chlorides, carbonates, soaps, and other salts of weak or volatile acids into sulfates. Any excess beyond the required quantity will attack the organic matter when the water is removed.

PRODUCTION OF ETHER-SOLUBLE MATTER. Combined fatty acid radicals are present in many carbohydrates (*8*), in some proteins (*5*), and in the complex lipides, especially the

TABLE I. WEIGHT LOSSES

(500 ml. of water evaporated in presence of grease samples)

Grease	Weight Taken	Final Recovery	Gain or Loss
	Mg.	*Mg.*	%
Butter	96.1	96.8	+ 0.73
Tallow	84.8	84.8	0.00
Beeswax	90.8	90.5	− 0.33
Lard	85.2	81.8	− 3.99
Grease from raw sewage sludge	115.5	100.2	−13.25
Grease from digested sewage sludge	126.5	108.1	−14.55
Soap acids from Ivory soap	118.5	101.2	−14.60
Sewage skimmings grease	83.1	67.9	−18.29
Sewage skimmings grease	87.8	65.8	−25.06

Table II. Insolubilizing of Grease by Heating 24 Hours in Presence of Air

	Initial Hexane-Soluble	Loss by Vaporization	Insoluble Residue	Final Hexane-Soluble
	Per cent of initial weight			
Grease from raw sludge	100	26.92	7.29	65.79
Grease from digested sludge	100	15.27	1.06	83.67
Fish oil	100	1.51	61.72	36.77
Tallow	100	5.54	13.42	81.04

relatively abundant phospholipides. In such combinations they exhibit none of the characteristics of grease.

A notable example is egg yolk, which, according to nutritional tables, is about one-half water, one-third fat, and one-sixth protein, yet it is not greasy, as the fatty material is combined in the form of the phospholipide lecithin. In food analyses it is customary to subject samples to acid hydrolysis, for only in this way is it possible to separate all the material which is fat, as judged from the nutritional viewpoint (*3*). The sanitary engineer, however, is generally not concerned about these chemically combined fats, since they are not separable by physical means and do not impart greasy characteristics to the waste. Hence they should not be included in grease analyses.

If a sample of sewage or sludge is evaporated to dryness while in an acidified condition, the combined fatty acid radicals will be partially liberated, to an extent depending upon the acidity and other conditions controlling the intensity of hydrolysis. A sample of egg yolk yielded 28 per cent of grease (wet basis) when analyzed by the standard procedure for sludge (*2*).

Insolubilizing of Oils by Oxidation and Polymerization. Evaporation of sewage samples to dryness may require 24 hours or more, depending upon the sample size and the method used. In this time there is ample chance for oxidation of fats, especially where they collect on the sides of the vessel above the receding water. The possible extent of this change was indicated by heating grease samples (about 100 mg. each) in dishes for 24 hours at 105° C.

Table II shows that considerable amounts of the greases became insoluble. It is evident that oxidation is potentially an important source of error.

Extraction of Nongreases. Glaring evidence of the extraction of nongrease is afforded by the presence of ash in burned samples of grease, obtained by extraction of dry sludges. Knechtges, Peterson, and Strong (*7*) report from 6 to 21 per cent ash in extracts. Soaps could not account for more than 3 per cent of ash, in view of the figures given for content of soap acids.

Ether extraction, by the authors, of samples of digested sludge which had been acidified and dried yielded a grease with 8.0 per cent of ash, whereas samples of the same sludge which were washed with acid and water to remove soluble inorganic salts before ether extraction yielded grease with an ash content of only 0.24 per cent. It is evident that the ash in the grease was due to the dissolving of inorganic salts by ether.

If organic solvents can extract such considerable amounts of inorganic matter, it is even more to be expected that they will extract other nongreasy matter, such as water-soluble organic acids, amino acids (which may be formed by the hydrolysis of proteins), amine salts, sugars, gums, resins, rubber, hydrocarbons of the naphthalene type, solid chlorinated hydrocarbons, sulfur, etc. The reporting of 0.25 and 0.3 per cent of nitrogen in grease samples by Knechtges, Peterson, and Strong (*7*) suggests the extraction of amino compounds.

Errors due to extraction of nongreases have also been commented on by Gehm and Trubnick (*6*).

Variation of Results with Various Solvents. "Standard Methods" (*2*) permits use of petroleum ether, ethyl ether, or chloroform (solvent to be stated). In sludge analyses chloroform gives results averaging 75 per cent higher than petroleum ether. Ethyl ether and most other solvents are ranged between these two in extractive power. Until a solvent is specified which can be shown to give a true measure of the content of grease in the appropriate sense of the word, there must remain a large element of uncertainty in the results.

Errors. When grease dissolved in the organic solvent is dried preparatory to weighing, three errors may arise: incomplete removal of solvent, oxygen absorption by the grease, and evaporation of grease. These errors are considered in detail in the next section.

Perfection of Procedure for Removing Solvent from Extracted Grease

In the course of research on the method of determining grease, it became evident that extraction methods could not be satisfactorily compared until accurate procedures were devised for drying and weighing the grease. Therefore, attention was directed first to that problem.

If considerable amounts of low-boiling oils are present and it is desired to include them with the oil or grease, extraction with a volatile solvent cannot be used. The American Petroleum Institute has adopted a procedure for oil-field waste water which involves partial distillation of the sample, collection of the distillate, separation of distilled oil by gravity, and volumetric measurement. A procedure of this sort must be used whenever volatile oils are of significance, since there is no practical way to remove solvent from such oils. But in analyses of most sewages and industrial wastes it is sufficient to determine those oils and greases which are heavy enough to permit their easy separation from volatile solvents. Since any grease will vaporize to some extent if heated for a long time, it was necessary to determine what technique would just remove the solvent with the minimum of vaporization or oxidation of the grease.

The grease may be dried and weighed in either flasks or dishes. Flasks have been chosen in this work because they permit recovery of solvent, are easier to manipulate, and occupy less space in the desiccator.

In order to determine the effect of time of heating on the weights of grease samples under conditions prevailing in analyses, a series of samples was added to 125-ml. conical flasks, which were then heated for various successive intervals of time at 105° C. The results, as given in Table III, show initial increases of weight in most cases, followed by decreases. The increases may be accounted for by absorption of oxygen, as was demonstrated by four series of tests, using lard and fish oil. Pairs of samples were weighed into flasks. One flask of each pair was kept filled with an atmosphere of inert gas, while the others were open to the air. The four samples exposed to air gained an average of 0.99 per cent when heated for one hour, while the greases protected by inert gas lost an average of 0.10 per cent.

A correct weight might be obtained at the time when the absorption of oxygen has just been offset by vaporization, but this time will vary with the nature of the grease, the size of the sample, the type of vessel, and other factors. It is better to minimize the heating period, so as to get a weighing before much oxygen has been absorbed.

In using a short heating time, removal of the solvent becomes critical. When flasks are heated on a steam bath until the solvent is apparently gone from the grease, the flask still remains filled with vapor, which will condense when cooled.

Table III. Changes in Weight of Grease at 105° C.

Grease	Weight Taken	Gain or Loss after Total Heating Times as Shown						
		5 min.	15 min.	60 min.	3 hours	8 hours	24 hours	100 hours
	Mg.	%	%	%	%	%	%	%
Mackerel oil	333.5	Zero	+0.12	+0.63	+1.80	....	+0.45	− 2.85
Herring oil	312.6	−0.10	+0.10	+1.79	+3.20	....	+1.92	− 3.17
Sewage skim	311.1	−0.74	−1.60	−2.06	−2.76	....	−5.27	−11.60
Tallow	312.9	+0.16	+0.35	+0.38	+0.45	+0.89	+0.16	−13.77
Beeswax	307.8	+0.07	+0.29	+0.32	+0.26	+0.26	+0.36	−16.25
Lard	305.3	+0.10	+0.16	+0.72	+1.15	+1.64	+0.23	−16.43
Butter	316.2	Zero	+0.22	+0.38	+0.44	+0.98	+0.73	−18.05
Soap acids	325.8	+0.06	0.00	−0.55	−0.92	....	−7.77	−36.82

If the flask is transferred to the oven without removing this vapor, a heating period of an hour is required to ensure that the vapor will diffuse out of the flask. A more satisfactory procedure is to remove the vapor before transferring to the oven. This may be done by tipping the flask to allow the vapor to flow out, or by blowing dry air or gas through the flask while it is on the steam bath, using a volume of air about ten times the volume of the flask.

In order to determine completeness of removal of solvent, pairs of flasks containing weighed quantities of grease (around 100 mg.) were run in parallel. Hexane was added to one of each pair of flasks; these were then placed on the water bath to vaporize the hexane. (The word "hexane" is here used to refer to a petroleum fraction consisting of a mixture of isomeric hexanes.) The vapor was blown out with ten volumes of gas; the flasks were transferred to the oven for various times, cooled, and weighed. Flasks which did not receive hexane were given the same heating in the oven, then cooled and weighed. Results are shown in Table IV.

In the case of herring oil, lard, and butter, the results at 10 minutes show evidence that a trace of hexane remained with the grease. At 15 and 20 minutes, results are somewhat erratic, but show no significant evidence that any hexane remained. With the sewage greases and soap acids, all samples showed losses, and in the cases of skimmings and soap acids the losses tend to be greater in those samples which had received hexane.

There seems to be no simple procedure to remove the solvent without some loss of volatile constituents from the sewage greases. It seems best to choose a procedure which will eliminate the added solvent and give reproducible results, ignoring the small amounts of volatile oils which are inevitably lost.

The ideal heating time will depend upon the solvent used and upon the quantity and kind of grease. Any specified time will be arbitrary and somewhat a matter of opinion, and subject to change when the procedure is applied to different kinds of materials. When using a solvent of boiling point similar to hexane, in the analyses of sewage or sludge samples yielding up to 500 mg. of grease, it seems reasonable to recommend heating for 15 minutes.

When using dishes the rates of vaporization are higher, as may be noted by comparing corresponding data of Tables II and III. A few tests indicated that if a grease solution in a dish is evaporated on a steam bath and heated for 5 minutes after the solvent is no longer visibly present, no additional heating in the oven is required.

The time in the desiccator also influences the results, as grease weights generally increase with time. With grease from sewage skimmings the increases were around 1 per cent in 48 hours. Fish oil samples sometimes increased as much as 4 per cent overnight. It is recommended that the time in the desiccator be between 1 and 3 hours.

Extraction Procedures

General Principles. Having determined a method for evaporating the solvent and weighing the grease, attention was directed to the extraction procedure. After trying a number of variations of the usual routine, it was concluded that the only hope of securing results of satisfactory accuracy lay in extracting the sample without evaporating the water. This plan is referred to as the "wet method", in contrast to the prevailing "dry methods". [Extraction in the wet state is an established method for analysis of oil-field waste water (*1*) and for milk (*4*), but as far as the authors are aware it has not previously been applied to analysis of sewage sludges and similar wastes.]

The wet method essentially involves the following steps:

Acidify the sample.

Shake thoroughly with successive quantities of solvent until removal of the grease is complete. Transfer the portions of solvent through a filter to a suitable receiving vessel.

Evaporate the solvent, heating just sufficiently to ensure complete removal of solvent. Cool in a desiccator and weigh after 1 to 3 hours.

Within the above outline, any variations of detail which seem reasonable to an experienced chemist and which are carried out with due attention to the requirements of quantitative analytical technique are permissible. The results of the wet method are not appreciably influenced by minor modifications of procedure.

Compliance with the general directions leads to certain difficulties, notably that of separating the solvent after shaking. Methods devised to accomplish this are set forth in the following suggested procedures. Procedure I is suitable for all types of sewage, sludges, and other fluid materials. No. II may be used with samples which do not form stubborn emulsions, and by proper manipulation, it may be used for sewage.

Wet Extraction, Procedure I. (1). The amount of sample required for a test varies with the nature of the waste. It is generally desirable that the yield of grease be between 50 and 500 mg. Sewage samples of 800 to 2000 ml. have been found convenient, but where heterogeneity of the material makes large samples desirable, volumes as large as 4000 ml. may easily be analyzed by using vessels of suitable size. In the case of sewage sludges, samples containing 1.0 to 5.0 grams of dry solids are usually satisfactory, but samples with 18 grams of dry solids have been analyzed without difficulty.

It is generally desirable that the sample be delivered in a glass-stoppered bottle containing the amount of material suitable for one analysis, so that any grease adhering to the vessel may be washed into succeeding apparatus.

2. Acidify the sample, using 5 ml. of 1 to 1 sulfuric acid. (For samples larger than 1000 ml., or for very alkaline materials, appropriately larger amounts of acid should be used.) Transfer to a separatory funnel of suitable size having a standard-taper ground mouth. (In the case of samples too large for available

Table IV. Completeness of Removal of Solvent from Grease

Grease (Approximately 100-Mg. Sample)	Gain or Loss in Weight of Grease					
	10 Minutes' Heating		15 Minutes' Heating		20 Minutes Heating	
	No hexane	Hexane added	No hexane	Hexane added	No hexane	Hexane added
	%	%	%	%	%	%
Herring oil	+0.66	+1.34	+0.30	+0.81	+0.95	+1.21
Lard	−3.24	+0.44	+1.13	+0.69	+0.97	+0.69
Butter	−0.36	+0.59	−0.07	+0.44	+0.07	+0.37
Grease from raw sludge	−2.42	−2.32	−2.42	−2.32	−3.00	−2.32
Soap acids	−0.74	−2.96	−0.66	−2.96	−0.17	−3.31
Grease from sewage skimmings	−1.77	−2.18	....	....	−3.60	−2.89

Table V. Grease Removed by Successive Extractions of Sewage and Raw Sludge

Extraction No.	Grease Recovered: Weight	Grease Recovered:	Per cent of total recovery
	Mg.	*P. p. m.*	
Sewage (Procedure II), Hexane Solvent			
1	36.8	47.6	82.5
2	7.0	9.1	98.2
3	0.8	1.0	100.0
4	0.0	0.0	100.0
Raw Sludge (Procedure I), Hexane Solvent			
1	262.8	13,140	79.23
2	46.3	2,315	93.19
3	12.9	645	97.08
4	6.7	335	99.10
5	2.8	140	99.94
6	0.2	10	100.00
7	0.0	None	100.00
8	0.0	None	100.00
Isopropyl Ether Solvent[a]			
9	4.9	245	....
10	3.7	185	....
11	1.8	140	....
12	1.2	60	....
13	0.2	10	....
14	0.0	None	....
15	0.0	None	....

[a] Following the eighth extraction, isopropyl ether was substituted for hexane, and the extraction continued.

funnels, the extraction may be carried out in bottles or other glass-stoppered apparatus.) If the entire contents of the sample vessel are being analyzed, rinse the containers out with a little solvent, adding the rinsings to the funnel. Add to the funnel 75 to 150 ml. of solvent which has been distilled in all-glass apparatus. Use proportionally larger amounts for samples larger than 1000 ml.

3. Shake the funnel vigorously for 2 minutes. Thin very thick sludges with water to facilitate this extraction step.

4. Connect the mouth of the funnel to a vertical condenser having a standard-taper ground-glass connection, and lower the assembly into a water bath maintained at a temperature of 90° to 100° C. (Cork connections may be substituted for ground-glass joints with introduction of scarcely appreciable errors.)

5. Reflux briskly until the emulsoid mixture has separated into its component parts. This usually requires from 15 to 30 minutes. It is desirable that the ebullition be principally from the top of the water layer. If the boiling occurs from the bottom of the funnel, the emulsion may not break.

6. Disconnect the funnel and cool for 5 or 10 minutes, preferably with water, set up a filter over a weighed conical flask of 125- to 250-ml. capacity, and transfer the clear solvent solution to the filter, using a 25-ml. pipet fitted with a 30-ml. rubber bulb. In order to avoid spilling drops of the solvent solution, the mouth of the separatory funnel should be held over the filter. Solvent must not be drawn into the rubber bulb.

To facilitate complete removal of the solvent layer, the water may be withdrawn from the bottom of the funnel, and then returned to the funnel after the solvent layer has been removed.

7. Add 50 to 100 ml. of solvent to the funnel and repeat operations 3 to 6. If desired, all or part of the water may be dis-

Table VI. Demonstration of Wet Method

Laboratory	Procedure	Solvent	Grease Content *P. p. m.*	Average Difference from Mean *P. p. m.*	*%*
Sewage 1 (Screened)					
A	II	Isopropyl ether	189		
B	II		191		
B	II		188	1.6	0.84
B	II		192		
B	II		192		
			Av. 190		
A	II	Benzene	196	...	...
A	II	Chloroform	194	...	...
Sewage 2 (Screened)					
A	II	Hexane	29		
B	I		31		
B	I		29		
B	I		33		
B	I		32		
B	I		31	0.91	2.9
B	I		30		
B	I		31		
B	I		31		
B	I		32		
B	I		30		
			Av. 31		
A	II	Isopropyl ether	40	...	...
Sewage 3 (Unscreened)					
B	II	Hexane	133	3.5	2.7
B	II		126		
			Av. 130		
A	A. P. H. A.		105		
A	A. P. H. A.		104	...	...
Sewage 4 (Unscreened)					
A	II	Hexane	67		
A	II		75		
A	II		69		
A	II		70		
A	II		72	2.4	3.4
B	I		67		
B	I		73		
B	I		73		
B	I		73		
			Av. 71		
Sewage 5 (Unscreened)					
A	I	Hexane	197	...	...
A	A. P. H. A.		135		
Sewage 6 (Unscreened)					
A	I	Hexane	127	...	...
A	A. P. H. A.		108		
Sewage 7 (Unscreened)					
A	I	Hexane	82	...	...
A	A. P. H. A.		59		
Sewage 8 (Unscreened)					
A	I	Hexane	92	...	...
A	A. P. H. A.		57		
Sewage 9 (Unscreened)					
A	I	Hexane	127	...	...
A	A. P. H. A.		101		
Sewage 10 (Unscreened)					
A	I	Hexane	48	...	...
A	A. P. H. A.		32		
Raw Sewage Sludge 1 (Screened)					
A	I	Isopropyl ether	17,560		
B	I		16,930	310	1.8
B	I	Ethyl ether	16,800		
			Av. 17,100		
A	I	Hexane	16,480		
A	I		16,590		
B	I		16,040	306	1.9
B	I		15,620		
B	I		15,950		
B	I		15,760		
			Av. 16,070		
A	I	Chloroform	16,790	270	1.6
B	I		16,250		
			Av. 16,520		
A	A. P. H. A.	Hexane	16,060		
Raw Sewage Sludge 2 (Unscreened)					
A	I	Hexane	14,110	55	0.39
A	I		14,220		
			Av. 14,165		
Raw Sewage Sludge 3 (Unscreened), 7.62% Solids					
A	I	Hexane	18,150	105	0.58
A	I		17,940		
			Av. 18,045		
A	A. P. H. A.		17,250	1060	...
A	A. P. H. A.		15,130		
			Av. 16,190		
Digested Sewage Sludge 1 (Unscreened), 8.6% Solids					
A	I	Isopropyl ether	8650	115	1.3
B	II		8420		
			Av. 8535		
B	II	Benzene	8600	...	...
B	II	Petroleum ether	7530	...	...
Digested Sewage Sludge 2 (Unscreened)					
A	I	Hexane	4420	40	0.90
A	I		4460		
			Av. 4440		

carded after the second extraction, since the remaining grease will be almost entirely in sludge solids and residual solvent. Make as many subsequent extractions as required to accomplish practically complete removal of the grease. For sewage, three or four extractions are sufficient, but sludges may require five or six extractions.

8. After all solvent layers have been transferred to the flask, wash the filter thoroughly with fresh solvent.

9. Separate the solvent from the fatty matter by a source of heat which cannot rise above 100° C. Either a water or steam bath is convenient. The flask may be connected to a condenser for recovery of the solvent, using ground-glass connections.

10. When no more liquid solvent is visibly present, introduce a jet of dry air or gas into the flask while it is still on the heating bath. This serves to displace the heavy vapor. The amount of air or gas used should be about ten times the volume of the flask. Use of larger volumes increases errors due to vaporization of grease.

11. Place the flask in a drying oven at 100° to 105° C. for 20 minutes, transfer to a desiccator, and weigh when cool. In order to minimize absorption of oxygen, the time in the desiccator should not exceed 3 hours. As a check on the completeness of the drying of the grease, reheat the flask for 10 minutes and reweigh. If the grease does not lose more than 2 per cent of its weight as a result of this reheating, the first weighing should be considered correct.

Procedure II. (1) Prepare the sample as in Procedure I, steps 1 to 3. For samples such as sewages, which tend to form emulsions, the shaking must not be too vigorous.

2. Allow the mixture to stand for 30 minutes, then draw off the lower layer of turbid water, which should separate into a second separatory funnel. Add 50 ml. of solvent to this second funnel and shake as before.

3. Returning to the first funnel, a layer composed of an intimate mixture of water, solvent, and solids will be found, which may be covered with a clear grease-bearing layer of solvent. Shake the mixture vigorously. This will generally cause solvent to separate and the residue to become granular. If no considerable separation occurs, add 50 to 100 ml. more of solvent and again shake well. If at this stage a resistant emulsion remains, continue the test by Procedure I.

4. When the solvent layer has clarified sufficiently, transfer it to a tared conical flask through a filter, as in Procedure I.

5. When the second separatory funnel has stood for a suitable time, withdraw the aqueous layer and discard it. Transfer solvent and emulsoid layer to the first funnel, rinsing with a few milliliters of fresh solvent. Shake the combined mixture again and allow it to stand until clear solvent can be transferred to the filter.

6. Make subsequent extractions of the residue in the separatory funnel by adding quantities of solvent at least twice the volume of the emulsoid mixture and shaking as before. Withdraw progressively any water which separates and discard after each shaking. The number of extractions will vary with the volume of the residue and the nature of the sample. In the case of sewage, a total of three or four extractions is sufficient.

7. Conduct the remaining operations as in Procedure I, steps 9 to 12.

Demonstration of the Method

Belief in the accuracy of the wet method rests in part upon experiments and in part upon theoretical considerations.

Table VI. Demonstration of Wet Method (*Cont'd*)

Laboratory	Procedure	Solvent	Grease Content	Average Difference from Mean	
			P. p. m.	*P. p. m.*	%
Digested Sewage Sludge 3 (Unscreened), 5.90% Solids					
A	I	Hexane	6920	40	0.58
A	I		6960		
			Av. 6940		
A	A. P. H. A.		6790	285	...
A	A. P. H. A.		6220		
			Av. 6505		
Meat Packing Waste Water (Screened)					
A	I	Petroleum ether	120	4.4	5.36
A	I		117		
A	I	Hexane	117		
A	I		109		
B	I		116		
B	I		117		
B	I		105		
			Av. 114		
A	I	Isopropyl ether	137	1.0	0.74
B	I		135		
			Av. 136		
B	I	Chloroform	137	...	...
Oil-Field Waste Water					
A	II	Benzene	71	2.0	2.9
B	II		67	...	...
			Av. 69		
A	II	Hexane	89	...	...
A	II	CCl_4 + ethyl ether			
Milk Sample 1					
A	II	Isopropyl ether	4093	35	0.86
A	II		4024		
			Av. 4058		
A	II	Chloroform	393	...	...
Milk Sample 2					
A	II	Isopropyl ether	3734	14	0.38
A	II		3708		
A	II	Ethyl ether	3723		
A	II		3694		
			Av. 3715		
A	II	Chloroform	3599	...	...
Fish Cannery Waste 1 (Screened)					
A	I	Isopropyl ether	274	4.3	1.6
A	I		273		
B	I		264		
			Av. 270		
A	I	Chloroform	281	7.7	2.8
A	I		276		
B	I		261		
			Av. 273		
Fish Cannery Waste 1 (Screened) (*Cont'd*)					
A	I	Petroleum ether	240	11.4	4.9
A	I		255		
B	I	Hexane	236		
B	I		211		
B	I		219		
			Av. 234		
Fish Cannery Waste 2 (Screened 20-Mesh)					
A	I	Hexane	896	11.3	1.28
A	I		893		
A	I		888		
A	I		851		
A	I		864		
A	I		874		
A	I		884		
A	I		884		
A	I		893		
A	I		879		
			Av. 881		
Fish Cannery Waste 3 (Screened 50-Mesh)					
A	I	Hexane	815	0.9	0.11
A	I		815		
A	I		817		
A	I		815		
A	I		816		
A	I		814		
A	I		816		
A	I		814		
A	I		817		
A	I		816		
			Av. 816		
Fish Cannery Waste 4 (Unscreened)					
A	I	Hexane	641	18.8	2.83
A	I		699		
A	I		654		
A	I		650		
A	I		676		
			Av. 664		
A	A. P. H. A.		777	154	...
A	A. P. H. A.		252		
A	A. P. H. A.		527		
A	A. P. H. A.		434		
			Av. 498		
			Weighted average		2.00

Experimental proof of a method would desirably include analyses of mixtures of known grease content. It is not practical to prepare such mixtures which could be considered identical in behavior with sewage or sludge, but a combination of substances was tried which may in some respects represent sewage. The preparation contained 1000 p. p. m. of diatomaceous earth, 1600 p. p. m. of shredded filter paper, 171.0 p. p. m. of lard absorbed on the filter paper, 113.1 p. p. m. of soap acids saponified with sodium hydroxide, and 350 p. p. m. of calcium hydroxide. Five analyses of this mixture by the wet method, Procedure I, gave results ranging from 280 to 286, averaging 283.2 or 99.67 per cent of the grease added.

The extent to which the grease recovered in analyses may be contaminated by inorganic substances may be judged by ashing. Three samples of grease separated by the wet method from sewage, raw sludge, and digested sludge showed, respectively, 0.43, 0.49, and 0.42 per cent of ash. (Hexane was used as a solvent.)

To be reliable, a method of analysis depending upon extractions must come to a sharp end point. By weighing separately the grease obtained in successive extractions, it is found that the wet method does exhibit a sharp end point. In fact, it appears that the grease remaining after each extraction is roughly proportional to the amount of solvent remaining. Table V shows tests of this sort for sewage and sludge. (In the analysis of sewage by Procedure II, the free water is discarded after the second extraction. The conclusion that all true grease is completely extracted from the water is based chiefly upon the fact that results obtained in duplicate analyses with and without discarding the water after two extractions differed by insignificant amounts.)

In sludge analyses, the amount of solvent remaining entrained in the sludge is variable, and generally greater than in sewage analyses: hence the extraction approaches completion irregularly and more slowly. Yet here also definite end points are indicated, as shown in Table V.

The importance of acid for decomposing the insoluble soaps was shown by analyzing raw sludge with and without acid. Results were, respectively, 6180 and 3280 p. p. m.

The possibility of acid hydrolysis of compounds such as lecithin was investigated by analyzing egg yolk. No grease was obtained by extracting in the cold, nor was any significant amount obtained after short periods of heating, but refluxing for 30 hours yielded 17 per cent of grease. Dry extraction by the A. P. H. A. method (*2*) gave 28 per cent of grease.

Data bearing upon the important questions of reproducibility of results, effect of choice of solvent, comparison of Procedures I and II, and comparison with the standard A. P. H. A. dry procedures, are presented in Table VI.

In many cases samples were analyzed independently in the laboratories of the authors (A and B, Table VI). Some samples, as indicated, were screened, the purpose being to facilitate division into representative subsamples.

The following observations can be made from the data of Table VI:

Reproducibility is satisfactory, the averages of the differences from the means being 2.0 per cent. (By standardizing all details of the procedure, as was done in the case of fish cannery waste No. 3, remarkably close agreement can be attained.)

There is a slight tendency toward higher results by Procedure I than by Procedure II, but the difference is usually of little importance.

Ethyl ether, isopropyl ether, chloroform, and benzene give results which differ by scarcely significant amounts but are considerably higher than results obtained with alkanes (petroleum ether, hexane). The differences, however, are not so great as have been reported in the case of dry methods.

In the analysis of sewage, using hexane as a solvent, the wet method gives results consistently higher than the A. P. H. A. method. With sludges the differences are in the same direction, but are smaller. In the case of fish cannery waste, it is impossible to secure reproducible results by dry methods. (The data given for fish cannery waste No. 4, A. P. H. A. method, are typical of several attempts to apply dry methods to this material.)

Since the variation of results with type of solvent is greater by the dry method than by the wet method, the observed relationship between results by the two procedures might be reversed if a solvent other than an alkane were used. Sewages and sludges from other origins might also show different relationships.

Considering again the sources of error in prevailing dry methods, as enumerated at the beginning of this article, it is clear that the change from a dry to a wet method has eliminated or reduced errors due to volatilization of grease when the sample is dried; resaponification of fatty acids from insoluble soaps; liberation of fats by hydrolysis of phospholipides, etc.; insolubilizing of oils by oxidation; and extraction of nongrease.

As to the inexactitudes which might be more serious in the wet than in the dry method, there are two worthy of mention: Acetic acid, and other lower members of the fatty acid series, if present, may be extracted to a considerable extent if ethers are used and to a slight extent with alkane solvents. The ease of extraction progressively increases with the higher homologs, so that butyric acid probably would be largely extracted even by alkanes. In analyses of sewage and raw sludge, this effect undoubtedly causes errors, which, however, are not likely to be important.

In some cases, the solvent may fail to contact and dissolve greasy particles which are surrounded by other material. Samples containing many large particles of solid matter may be broken up in some apparatus such as a food "liquefier" to reduce this source of error. That such inaccuracy is not likely to be serious in ordinary work is indicated by the following consideration:

Prolonging the time of shaking beyond that recommended in the procedures does not influence the test, and even the much more thorough contact between sludge particles and solvent in Procedure I, as compared with Procedure II, does not give significantly higher results.

The consistency of the results suggests that the extraction is not greatly dependent upon the variable chance of contact between solvent and grease particles.

Grease particles must be effectively surrounded by hard material which is preferentially wet by water rather than hexane in order to escape solution by the hexane.

In spite of these considerations, some grease does escape extraction, as, for example, on the inside of seeds. However, such sequestered grease is of relatively little concern to the sanitary engineer, for as long as the encasement remains unruptured, it does not impart greasy characteristics to the waste.

In judging the merits of methods, time requirements are important. Results by the wet method can be obtained in less than 8 hours, which is considerably under the time required for the A. P. H. A. dry method, but perhaps twice as much of the chemist's time is required. With adequate equipment, one chemist can conveniently analyze five samples per day by the wet method.

Choice of Solvent

A liquid of ideal solvent action should dissolve all substances having the sanitary significance of grease, including such water-insoluble materials as oils, fats, insoluble soaps (or the corresponding soap acids), waxes, and soft tars. Unfortunately, any solvent which will completely dissolve these substances will also dissolve some nongreases. Thus hexane, which gives lowest results of any of the liquids used, will dissolve such nongreaselike compounds as naphthalene, *p*-di-

chlorobenzene, *o*-toluidine, benzoic acid, etc., yet there is a soft brown tar in oil-field waste water which is insoluble in hexane.

As far as is known, chloroform and benzene will dissolve all materials which could be classed as grease, but these solvents also attack resins, dried paint, rubber, hard gums (such as chicle), etc. As noted previously, the results of grease analyses obtained with these solvents are higher than with alkanes.

When the material extracted by chloroform from sewage sludge was treated with hexane, there remained a black, powdery solid. This was resoluble in chloroform, had a density greater than unity, and was only slightly softened in boiling water. Clearly, such material is not grease. Meat-packing waste yielded a similar substance. Thus it appears, at least in the cases of sewage and meat-packing wastes, that the higher results obtained by chloroform were due chiefly to extraction of nongreases, rather than to more effective removal of greases. The same conclusion probably applies to benzene and the ethers.

The authors are of the opinion that alkanes should be used for analyses of sewage, sewage sludges, and most industrial wastes. A petroleum fraction approximating the hexanes in properties (or pure isohexane or *n*-hexane) is preferred to petroleum ether, since the latter product is variable in properties and has a boiling point undesirably low.

Oil-field water and possibly some other wastes may require a different solvent, but the choice should be based upon a study of the materials dissolved, rather than upon the assumption that the solvent giving highest results is best.

Summary

Many errors are inherent in those grease analysis methods which call for evaporating the water present in the sample. Procedures have been devised whereby the extraction is effected without this preliminary drying. Application of these procedures to a variety of sewages, sludges, and industrial wastes gave results with differences from the means averaging 2.0 per cent. Results are much less influenced by variations of technique and of solvent and more accurately represent the true content of "grease" than is the case in "dry" methods.

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A Rapid Procedure for Determination of Carbonate

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THE simplest procedure for the determination of carbonate consists in addition of acid to the sample, removal of carbon dioxide by boiling, and titration of the excess acid. This procedure has very limited application, as it may be used only when the carbonate is all present as either carbonate or bicarbonate and when other acidic or basic substances are absent.

The modification of this procedure here described extends its use, so that it may be used more generally.

The total carbon dioxide is quantitatively precipitated as strontium carbonate and the solution is adjusted to neutral to phenolphthalein. A known amount of acid is added to liberate the carbon dioxide, which is then aerated from the solution, and the excess acid is titrated with alkali. If organic acids or bases are present they are either wholly or partially neutralized in the preliminary pH adjustment; after the addition of acid they are brought back to this same state of neutralization. Phosphate is precipitated as strontium phosphate, and after the addition of acid, reprecipitated in the back-titration. Iron is precipitated as its oxide and is titrated back to this form. When present in small amounts ammonia does not interfere; in large amounts it interferes with the phenolphthalein end point. Magnesium is precipitated as the hydroxide when the solution is first made alkaline and it dissolves slowly when the solution is neutralized. It interferes in so far as it is not dissolved during this neutralization.

This procedure is somewhat faster than others, especially when a number of samples are run at the same time, and it may be used when other procedures based on the formation of insoluble salts are not applicable. Thus, the procedure based on titration in the presence and absence of barium (*1*) is limited to alkaline solutions which do not contain phosphate or calcium. For the quantitative precipitation of carbonate an alkaline solution of strontium chloride is preferred to the more generally used barium hydroxide (*3*, *4*) because strontium carbonate is less soluble than barium carbonate and because the large excess of strontium decreases this solubility still further. The procedure (*2*) in which carbonate is precipitated as calcium carbonate, filtered off, and titrated cannot be used in the presence of other ions which form insoluble calcium salts.

Method

In each of four large 35 × 200 mm. test tubes place 10 cc. of carbonate-free 0.03 *N* sodium hydroxide and a few drops of phenolphthalein. [Carbonate-free alkali may most easily be prepared by measuring saturated sodium hydroxide (19 *N*) into slightly acidified boiled distilled water. To prepare the saturated sodium hydroxide, dissolve 100 grams in 100 cc. of water and allow the solution to cool and settle.] Add carbonate solution to two of the tubes, delivering the sample underneath the alkali to prevent loss of any free carbon dioxide. Add 10 cc. of 20 per cent strontium chloride solution to each tube. Mix the contents of the tubes, stopper, and allow them to stand for several minutes. The

Table I. Determination of Carbonate

Expt. No.	Composition of Unknown Solution[a] Total volume	0.1 M carbon dioxide	Other 0.1 M constituents	0.1 M Carbon Dioxide Recovered	
	Cc.	Cc.	Cc.	Cc.	%
1	80	0.30	None	0.291	97
2	15	1.50	None	1.50	100.0
3	25	25.00	None	25.04	100.2
4	5	4.00	None	4.02	100.5
5	5	4.00	0.3 KH_2PO_4	3.96	99.0
6	7	4.00	2.0 KH_2PO_4	3.96	99.0
7	6	4.00	1.0 $FeCl_3$	4.03	100.8
8	6	4.00	1.0 $MgCl_2$	3.96	99.0
9	10	4.00	5.0 $MgCl_2$	4.33	108.0
10	6	4.00	1.0 NH_4Cl	3.99	99.8
11	10	4.00	5.0 NH_4Cl	4.00 ± 0.2	100.0 ± 5
12	15	4.00	10.0 NH_4Cl	4.00 ± 0.4	100.0 ± 10
13	9	4.00	2.0 $MgCl_2$ 2.0 KH_2PO_4	3.96	99.0
14	7	4.00	1.0 $FeCl_3$ 1.0 KH_2PO_4	4.25	106.0
15	5	0.56	5 cc. urine	0.46	82
16	5	3.70	5 cc. urine	3.60	97
17	11	0.21	1 cc. blood	0.17	81
18	5	0.30	3 cc. 1.5% albumin	0.303	101

[a] In experiments 1 to 15 and 18, known amounts of sodium bicarbonate and other constituents were dissolved in water. Urine and blood samples (experiments 15 to 18) were analyzed by Van Slyke manometric technique.

solutions must remain alkaline. Adjust the reaction to just pink to phenolphthalein by slowly adding 0.1 N hydrochloric acid from a small-tipped buret. In order to avoid the accumulation of acid in any part of the solution, with consequent loss of carbon dioxide, stir each solution with a thin glass rod, bent and flattened on one end. Avoid breaking the surface of the liquid with the stirring rod and the addition of an excess of the acid.

Add 5 cc. of 0.1 N acid to each tube to dissolve the precipitated strontium carbonate. Aerate the contents of each tube rapidly for 4 minutes, using a Folin aeration tube (a tube perforated with several small holes). Titrate the excess acid with 0.03 N alkali. The difference between the titration values of the sample and the blank gives the amount of carbon dioxide in the sample. Since carbonate acts as a divalent acid, 1 mole of alkali is equivalent to 0.5 mole of carbon dioxide.

The amounts and concentrations of reagents used should be adjusted to the amount of carbonate and other buffering substances present in the sample. The values given in the above description are suitable for samples which contain from about 0.75 to 3.00 cc. of 0.1 M carbon dioxide, when little other buffering substance is present (experiment 2, Table I). When very little carbonate is present (experiment 1), use smaller amounts of reagents, a larger unknown sample, run blanks on an equal volume of carbon dioxide–free water, allow the reaction mixture to stand for 15 minutes before neutralization, and aerate with carbon dioxide–free air. With urine samples (experiments 15 and 16), it is convenient to measure 5 cc. of urine into 5 cc. of 0.3 N alkali, add strontium chloride, neutralize, add 10 cc. of 0.3 N acid in excess, and titrate with 0.1 N sodium hydroxide.

Discussion

This procedure can be used under widely different conditions, provided a number of precautions are observed. At no time previous to the aeration may the sample become acidic. When strontium chloride is added it reacts with bicarbonate or phosphate to form hydrochloric acid. The reaction mixture must contain sufficient alkali to neutralize this liberated acid.

When the excess alkali is neutralized, acid must be added slowly and the solution must be mixed continuously to avoid loss of carbon dioxide from the acidified surface of the solution. The alkaline tubes must be kept stoppered to prevent absorption of carbon dioxide from the air. The amount of absorption, even in stoppered tubes, depends on the amount the tubes are shaken. They may be kept under nitrogen, but nitrogen should not be run into the tubes while excess alkali is being neutralized because of danger of carrying off carbon dioxide liberated at the surface of the solution.

When phosphate is present it is sometimes reprecipitated somewhat slowly when the excess acid is titrated, resulting in a fading end point. This continues for only a minute or two and then gives rise to a stable end point. Neither iron nor phosphate interferes when present singly (experiments 5, 6, and 7), although they indirectly decrease the precision by requiring the use of larger amounts of reagents. When present together in the same solution (experiment 14) they may be precipitated as ferric phosphate in the preliminary neutralization and as ferric hydroxide and strontium phosphate after the excess acid is titrated. Since these forms contain different amounts of alkali, these substances may interfere when present together.

If a relatively large amount of magnesium is present (experiments 8, 9, and 13) some is precipitated as hydroxide when the solution stands in excess alkali. When the alkali is neutralized this dissolves slowly. It is sometimes necessary to readjust the solution to neutral several times over a period of about a half hour before a permanent end point is obtained. Although quantitative recovery may be obtained when magnesium is present, it increases the time required to run the determination to such an extent that it should be considered an interfering substance.

When applied to acid urine samples which contain very small amounts of carbonate, the procedure is not accurate. This is at least partly due to the fact that the carbonate is so small a fraction of the total buffers present. With more alkaline urine samples which contain more carbonate (experiment 18), the results are within 4 per cent. When the method was used for the determination of carbon dioxide in blood serum, irregular results were obtained which were lower than values obtained by gasometric procedures. Egg albumin at about the same concentration as the proteins of blood did not interfere with the determination.

Table II. Determination of Carbonate in Limestone[a]

Sample No.	Composition of Limestone R_2O_3	$MgCO_3$	CO_2	Carbon Dioxide Determined Titrimetrically	
	%	%	%	%	%
2	5.83	40.15	42.78	42.88	42.95
10	4.9	34.7	42.89	42.95	.....
42	41.8	16.53	25.95	25.93	25.87
44	48.3	8.87	22.65	22.71	22.50
x			21.63	21.81	21.86

[a] Analyzed samples kindly supplied by H. V. Moyer, Chemistry Department, Ohio State University.

This general procedure may also be applied to the determination of carbon dioxide in solid materials. The data in Table II give analyses for limestones.

Weighed samples (0.28 gram) were treated with 1 cc. of 0.1 N sodium hydroxide, 5 cc. of 20 per cent strontium chloride, and a few drops of phenolphthalein. The mixtures were allowed to stand for a few minutes, so that any bicarbonate which might be present would be changed to carbonate. The reaction mixtures were then neutralized and treated with an excess of 25 cc. of 0.3 N hydrochloric acid. The mixtures were heated at 80° C. in a water bath and shaken at 5-minute intervals over a period of 30 minutes in order to dissolve the carbonate in the limestone. After they had cooled the tubes were aerated for a few minutes and the excess acid was titrated with 0.1 N alkali. The difference between this titration and a blank titration represents the amount of carbon dioxide in the sample.

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Proximate Analysis of the Heartwood and Sapwood of Some American Hardwoods

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SEVENTEEN years have elapsed since any intensive analyses of American woods have been undertaken. Since that time many of the methods have been revised, and new ones introduced, notably a reasonably rapid method for the evaluation of the total carbohydrate fraction in wood (holocellulose). It was therefore deemed advisable to select the best of these methods and incorporate them into a new scheme of wood analysis which in reality is a modification of the original U. S. Forest Products Laboratory procedure as proposed by Schorger *et al.*

In contrast to the work of earlier wood analysts (*14*), the authenticity of the wood samples in all instances has been verified by means of microscopic identification. The leaves and fruit of each tree have been collected, identified, and are now on file at the Arnold Arboretum, Boston, Mass. Furthermore, the silvicultural characteristics, collection date, age, and height have been recorded for reference purposes.

Analytical Methods

While the methods generally have been described fully in the literature, they are outlined briefly in the interest of better interpretation of the results.

All wood samples were taken from the bole of each tree at a point approximately 20 feet above the ground. This particular section was chosen because strength tests at the U. S. Forest Products Laboratory (*19*) have shown this portion to be most representative of the specimen as a whole. The wood was then chipped and reduced to sawdust in a Wiley mill. Material which passed a 60-mesh sieve but was retained on an 80-mesh screen was chosen as the optimum particle size for analysis.

The determination of ash content and solubility in water, alcohol-benzene, and ether conformed with standardized procedures (*19*). Acetyl was estimated by a slightly modified procedure of Freudenberg's (*4*) method, which depends upon the transesterification in alcohol of the acetyl constituents by *p*-toluenesulfonic acid. This was followed by titration, after saponification of the ethyl acetate removed by distillation. Estimation of the methoxyl content was accomplished according to the well-known method of Zeisel (*21*). The lignin content was determined by the method of Klason as modified by Ritter, Seborg, and Mitchell (*17*). Inasmuch as the accuracy of the results obtained compares favorably with those of other well-known methods, and is most acceptable where many determinations are to be made, it was thought advisable to adopt this procedure for the present study.

The pentosan and cellulosan content of the wood was determined by the volumetric potassium bromate-bromide method of Powell and Whittaker (*12*). Kline and Acree (*9*) as well as Iddles and Robbins (*7*) have reported that this procedure consistently gives accurate results which compare favorably with the well-known gravimetric methods. For this reason and because of the rapidity with which results may be obtained, the method was selected for use in the present study.

For clarification of Table I, it should be pointed out that the term "cellulosan" is used to designate the pentosan associated with the cellulose. It was first proposed by Hawley and Norman (*6*), and has been generally accepted by workers in the field of wood chemistry.

Cellulose was estimated by the Cross and Bevan method as modified by Ritter and Mitchell (*16*). The quantitative determination of the total carbohydrate content of the wood, termed holocellulose, formulated by Ritter and Kurth (*10*, *15*) depends on the isolation of this fraction by alternate treatment of extractive-free wood with chlorine gas and alcohol-pyridine solution until the weight loss equals that of the lignin present in the original wood. Since it was felt that bleaching might possibly alter some of the constituent groups in this residue all analyses were made on unbleached holocellulose.

The analytical results presented in Table I represent the average of three determinations.

Discussion

HOLOCELLULOSE DETERMINATION. During the course of the present investigation approximately 75 different holo-

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TABLE I. ANALYSIS OF AMERICAN WOODS

[Summary of analytical results in percentages of oven-dry (105° C.) samples]

Species	Ash	Solubility: Cold water	Solubility: Hot water	Solubility: Alcohol-benzene	Ether	Acetyl	Methoxyl	Pentosan	Lignin[a]	Cellulose	Cellulosan	Holocellulose	In Holocellulose: Acetyl	In Holocellulose: Methoxyl	In Holocellulose: Pentosan
													(*Per cent on original wood*)		
Big tooth aspen															
Sapwood	0.26	2.70	3.13	2.41	1.02	5.48	5.27	23.33	16.33	62.67	14.45	78.01	5.17	0.65	22.51
Heartwood	0.33	1.36	0.99	2.13	1.03	6.07	5.35	23.75	16.92	64.42	15.78	80.00	5.05	0.72	22.98
Beech															
Sapwood	0.31	2.33	2.17	1.37	0.20	7.13	6.28	25.55	20.61	60.83	17.88	76.20	5.15	0.95	24.46
Heartwood	0.57	0.23	0.43	0.96	0.57	6.05	6.44	24.49	22.26	60.71	18.33	76.85	4.76	1.24	23.52
Sugar maple															
Sapwood	0.32	0.81	2.08	1.31	>0.10	6.56	6.34	22.78	20.33	60.39	16.02	72.26	5.12	0.83	22.67
Heartwood	0.84	0.52	1.20	1.22	0.89	4.94	6.50	22.98	21.79	60.27	14.25	75.96	4.05	0.97	22.57
Fire cherry															
Sapwood	0.17	1.96	4.03	2.97	0.72	7.54	4.61	26.16	12.27	59.98	14.32	80.96	7.51	1.06	25.01
Heartwood	0.41	0.67	2.48	1.18	0.47	6.77	4.77	27.32	14.82	58.26	14.93	81.58	5.23	1.16	25.65
Paper birch															
Sapwood	0.24	1.28	2.39	3.31	0.79	7.12	6.10	28.77	17.56	55.58	17.98	76.61	6.01	0.97	28.45
Heartwood	0.21	1.12	2.15	6.44	2.19	7.60	5.75	28.64	19.61	50.96	13.97	70.85	5.29	0.95	26.93
Yellow birch															
Sapwood	0.11	1.20	1.30	0.97	0.36	8.79	6.01	26.89	18.56	59.36	19.27	79.50	6.13	1.31	26.49
Heartwood	0.50	0.93	1.29	1.89	0.30	6.11	6.04	26.87	20.19	58.37	16.99	76.45	5.81	0.93	24.94

[a] Ash-free.

Table II. Summative Analysis of American Woods[a]

Species	Ash	Solubility: Hot water	Solubility: Alcohol-benzene	Lignin	Holocellulose	Total
	%	%	%	%	%	%
Big toothed aspen						
Sapwood	0.26	3.13	2.41	16.33	78.01	100.14
Heartwood	0.33	0.99	2.13	16.92	80.00	100.37
Beach						
Sapwood	0.31	2.17	1.37	20.61	76.20	100.66
Heartwood	0.57	0.43	0.96	22.26	76.85	101.07
Yellow birch						
Sapwood	0.11	1.30	0.97	18.56	79.50	100.44
Heartwood	0.50	1.29	1.89	20.19	76.45	100.32
Paper birch						
Sapwood	0.24	2.39	3.31	17.56	76.61	100.11
Heartwood	0.21	2.15	6.44	19.61	70.85	99.26
Fire cherry						
Sapwood	0.17	4.03	2.97	12.27	80.96	100.40
Heartwood	0.41	2.48	1.18	14.82	81.58	100.47
Sugar maple						
Sapwood	0.32	2.08	1.31	20.33	76.26	100.30
Heartwood	0.84	1.20	1.22	21.79	75.96	101.01

[a] Results in percentages of oven-dry (105° C.) wood.

cellulose residues were isolated. The outstanding objection to the holocellulose determination is the lack of a definite end point indicating complete removal of all lignin. At present the results obtained are entirely dependent upon the accuracy of the lignin determination, and since in the latter case there is no one method in use today which has received the unanimous endorsement of all workers in this field, the empiricism of the holocellulose procedure may be seriously questioned. It would be highly advantageous, therefore, to modify the determination in such a manner that it would be independent of other procedures, thus increasing both its efficiency and accuracy[2].

Summation of Proximate Groups. As a means of accounting for the total wood substance analyzed a résumé of the analytical data is given in Table II. In this summary it has been assumed that the total mineral content of the wood is included under "ash", whereas any waxes, fats, etc., are accounted for under "alcohol-benzene solubility". Certain of the simpler carbohydrates and water-soluble dyes will be removed by the hot-water extraction. The remaining residue is thus composed of a noncarbohydrate fraction, designated as lignin; and a carbohydrate fraction, designated as holocellulose. As shown in Table II, the summation equals 100 per cent (±1 per cent) for each species analyzed.

Relation of Sapwood and Heartwood Constituents. Ritter and Fleck (*14*) have reported that in the analysis of softwoods certain definite conclusions may be drawn concerning the relationship between the various constituents of the sapwood and heartwood, although they add that it is difficult to generalize in the case of the hardwoods. These authors also observe that the acetic acid which results from the hydrolysis with mineral acid is consistently higher in the sapwood than in the heartwood of both softwoods and hardwoods.

The results of the present investigation fail to confirm the conclusions of these investigators. That the relationship suggested by Ritter and Fleck fails to exist is not unusual when it is considered that certain vital factors such as age, growing conditions, and habitat are responsible for the amount and distribution of these constituents.

The data of the present study, however, do permit certain generalizations to be drawn which must be interpreted strictly as such, and not as conclusions.

With but one exception (paper birch) the percentage of ash is considerably lower in the sapwood than in the heartwood fraction.

In every case the hot- and cold-water solubility is greater in the sapwood than in the heartwood.

With the exception of the two birches, alcohol-benzene solubility is also higher in the sapwood of the remaining four species.

Of the woods investigated no generalizations can be drawn relative to a specific distribution of ether-soluble residue, pentosans, or holocellulose.

The acetyl content of the sapwood is greater than that of the heartwood in four of the six species. Aspen and paper birch are exceptions.

With the exception of paper birch, the methoxyl residue is greater in the heartwood fraction.

Inasmuch as it is generally agreed that methoxyl is for the most part associated with lignin, it is not surprising that the heartwood of every species contains more lignin than the sapwood.

In contrast, with one exception (aspen), the cellulose content of the sapwood of all species is higher than that of the heartwood.

Any generalization as to the distribution of the cellulosan fraction between sapwood and heartwood must be made with extreme caution, because of the possible formation of small amounts of furfural-yielding degradation products during isolation of the cellulose. With such reservations in mind, it is observed that in four of the woods studied the cellulosan content is higher in the sapwood than in the heartwood. Aspen and beech are exceptions.

Distribution of Pentosan, Methoxyl, and Acetyl Groups. The results of the present investigation provide some interesting observations regarding the distribution of pentosan, methoxyl, and acetyl groups between the carbohydrate (holocellulose) and noncarbohydrate (lignin) portions of the wood. By calculation it was found that 98 per cent of the pentosan residues present in the original wood could be accounted for in the holocellulose fraction of each species. This figure is in excellent agreement with the results of Ritter and Kurth (*15*), who report 96.6 per cent of the total pentosans to be present in the holocellulose isolated from maple wood.

The results given in Table I also show that an average of 16 per cent of the total methoxyl content of the wood may be accounted for in the carbohydrate fraction.

Earlier investigators, Benedikt and Bamberger (*1*) and Dore (*3*), have stated that methoxyl is entirely associated with the lignin. In contrast, Ritter and Kurth (*15*) report that 15.6 per cent of the total methoxyl is present in the holocellulose residue; and Hägglund and Sandelin (*5*) have shown that 12 per cent of the methoxyl content of wood is present in the carbohydrate portion. Added confirmation is supplied by the work of O'Dwyer (*11*) and Schmidt *et al.* (*18*). Thus, the bulk of experimental evidence at present disproves the former conception that all of the methoxyl residues in wood are associated with the lignin.

Cross and Bevan (*2*), Pringsheim and Magnus (*13*), and Klason (*8*) claim that all of the acetyl residues are associated with the lignin. Dore (*3*), however, believes they are joined with the cellulose. Ritter and Kurth (*15*) also claim to disprove the earlier belief by reporting that the total acetyl groups of the original wood of sugar maple may be accounted for in the holocellulose fraction. However, Hägglund (*5*) reports a reduction of 40 per cent of the acetyl content of holocellulose as compared to that in the original wood. Kurth and Ritter have raised the objection that Hägglund's use of alkaline calcium hypochlorite bleach may possibly have resulted in the removal of some of the acetyl residues, thus accounting for the low results reported.

The results of the present study, which covers a total of twelve separate sets of analyses of six different hardwoods including sugar maple, indicate that an average of 81 per cent of the total acetyl is accounted for in the holocellulose. Un-

[2] Since the completion of this study, Van Beckum and Ritter (*20*) have published their chlorine–alcoholic ethanolamine method, which is a better procedure than the use of chlorine-alcohol-pyridine in that a sharp end point is said to be obtained upon removal of the last traces of lignin.

fortunately, these results cannot be interpreted as substantiating Hägglund, because examination of Table I will show that in the cases of aspen sapwood, fire cherry sapwood, and yellow birch heartwood the acetyl content of the holocellulose represents 94, 99, and 98 per cent, respectively, of that in the original woods. Therefore, in three instances the results confirm those of Ritter and Kurth (*15*). Because of these contradictory results no clear-cut conclusion can be drawn regarding the location of the acetyl group. Possibly a portion of the acetyl groups in the other woods have been destroyed during the isolation of the holocellulose, owing to partial oxidation during chlorination and removal of the decomposition products during the pyridine-alcohol extraction.

Application of Results

In one instance the results of the present investigation have brought to light information which otherwise might have escaped attention. It was noticed that the lignin content of fire cherry was exceptionally low, thus predicting a high carbohydrate content. Evaluation of the cellulose content yielded an average figure of only 60 per cent. The difference between the cellulose and holocellulose contents suggested the presence of a considerable quantity of hemicellulose which was isolated and is now under investigation.

Acknowledgment

The authors are indebted to H. P. Brown, New York State College of Forestry, Syracuse, N. Y., for collection and identification of the woods analyzed.

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A Laboratory-Scale Flow Regulator

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EXPERIMENTS conducted in this laboratory involving the slow transport of gases have made necessary the use of a device for regulating their flow.

Although a few designs intended for laboratory use are reported in the literature (*1–4*), all possess certain limitations in regard to expense, simplicity, reproducibility, and personal supervision. To meet all these factors, the conditions of which were imposed by a problem under investigation, it was necessary to design a regulator.

The apparatus (Figure 1) is fashioned from the escapement wheel, *W*, and the lever, *L*, of a pendulum clock. A perforated strip of metal, *S*, 30 inches long and 0.75 inch wide, is welded to the end of the escapement lever. A weight, *M*, is then attached to the lower end of the metal strip, producing a typical pendulum with a relatively long period of vibration.

A drum, *D*, is fastened on the axis of the escapement wheel, and is then wound with a stout cord or wire.

A leveling bulb containing mercury or some other liquid is now suspended from the drum by the cord. The weight of the leveling bulb thus acts on the escapement wheel and provides the force necessary to keep the pendulum in motion. As the wheel turns and the drum unwinds, the leveling bulb is lowered slowly, reducing the pressure within a bottle to which the leveling bulb is connected and causing gas to flow into the bottle.

The purpose of the pendulum is to maintain a constant lowering of the leveling bulb. The weight of the bulb increases as it fills with liquid, but the effect on the pendulum is one of increased amplitude of vibration rather than shortening of the period.

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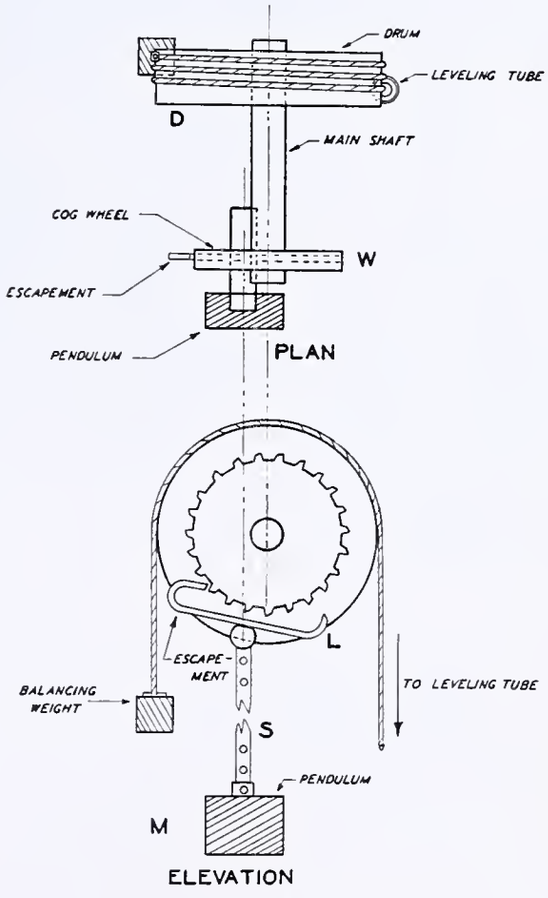

Figure 1. Flow Regulator

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Determination of Carotene and Cryptoxanthin in Yellow Corn

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YELLOW corn is an important source of vitamin A in feeds, even though the amount it contains is low when compared to good alfalfa and other hays of good quality. Such large quantities of yellow corn are usually fed that it may afford a considerable portion of the vitamin A potency an animal receives. Kuhn and Grundmann (*10*) in 1934 and Buxton (*2*) later reported that as much as 90 per cent of the active pigments in yellow corn may consist of cryptoxanthin. According to Kuhn and Grundmann (*9*) cryptoxanthin has one half the vitamin A potency of beta-carotene. Therefore, in order to determine by chemical methods the vitamin A potency of yellow corn and commercial feeds which contain it, it is necessary to determine both cryptoxanthin and beta-carotene.

Chromatographic methods have been used extensively to separate and identify carotinoid pigments in plant materials and have been applied to the determination of carotene and cryptoxanthin in yellow corn (*2*, *10*). The methods heretofore described are slow and complicated, and losses of pigments in the column have sometimes been so high that the final results obtained are doubtful. The purpose of the work reported here was to develop simple methods for determining the quantities of carotene, cryptoxanthin, and other pigments having vitamin A potency in yellow corn.

Chromatographic Method

In previous work Fraps, Kemmerer, and Greenberg have reported methods by which crude carotene solutions from alfalfa (*4*) and other dried hays and grasses, which contain some impurities, and solutions from tomatoes (*5*) and watermelon, which contain large amounts of lycopene, could be highly purified by shaking with magnesium carbonates activated in such a manner that they would adsorb large amounts of xanthophyll or lycopene but not carotene. A number of attempts were made to adapt these methods to the determination of carotene and cryptoxanthin in yellow corn but the desired separation could not be made. Then chromatographic methods were applied.

After some preliminary work, the following complete chromatographic method was found to be the most satisfactory:

The crude carotene was adsorbed by a column of magnesium oxide, and the bands of pigments were separated by washing and removed mechanically. The pigments were dissolved and the quantities determined. The magnesium oxide was of such quality that when 2.5 grams were shaken with 50 cc. of a solution of 2.0 parts per million of carotene in petroleum ether, 50 per cent of the carotene was absorbed. For the adsorption a glass tube was used 5 to 8 mm. wide and 15 to 20 cm. high, constricted at one end and surrounded by a condenser jacket cooled with ice water during the separation of the pigments. A small wad of cotton was placed in the constricted end of the tube and U. S. P. light magnesium oxide was put into the tube in 0.2-gram portions. Suction was applied and each portion of magnesium oxide was packed gently but firmly by means of a cork with a smooth surface, attached to a glass rod. Portions of magnesium oxide were added and packed until a column about 10 cm. long was obtained.

For the determination 25 grams of finely ground yellow corn were refluxed with 12 per cent alcoholic potassium hydroxide and the crude carotene was obtained in petroleum ether (Skellysolve F, boiling point 30° to 76.7° C.) as described in the A. O. A. C. method for carotene (*1*). The crude carotene solution was diluted to 200 cc. in a graduated flask and the amount of pigment, expressed as carotene, was determined with a photoelectric colorimeter. Then the solution was concentrated to about 25 cc. and placed on the column described above. The column was washed with petroleum ether until the bands of pigment had separated, which usually took about 4 hours. Each band of pigment was scraped from the column into a beaker, with a long wire flattened at one end, and was always kept covered with petroleum ether. The bands were separately extracted with petroleum ether containing 2 per cent ethanol, the extracts were washed with water to remove the alcohol, and if the solutions were cloudy they were washed once with dilute hydrochloric acid (1 to 100). The solutions were dried over anhydrous sodium sulfate and diluted to suitable volume in graduated flasks, and the amount of pigment (expressed as carotene) was determined with the photoelectric colorimeter.

Analyses by Complete Chromatographic Method

Seven bands of pigment were frequently formed in the chromatographic column.

(1) At the top of the column, for a few samples, was a small narrow band of orange pigment, which the authors termed "impurity". (2) Next was a wide orange band of cryptoxanthin, and then (3) a band of what Zechmeister and Tuzson (*13*) call neocryptoxanthin. Very close to and below the neocryptoxanthin band were two extremely fine bands (4), one red and the other orange. It was impossible to separate these bands quantitatively from the neocryptoxanthin by the mechanical technique used. In the work reported here they were included with the neocryptoxanthin. Next (5) was a light yellow band, which, because of its position in the column and because it was later found to possess vitamin A potency, the authors call K carotene. Next (6) was a sharply separated orange band of beta-carotene, and finally (7) a small orange band of alpha-carotene.

The alpha- and beta-carotene bands in the column were identified by means of the mixed chromatographic technique

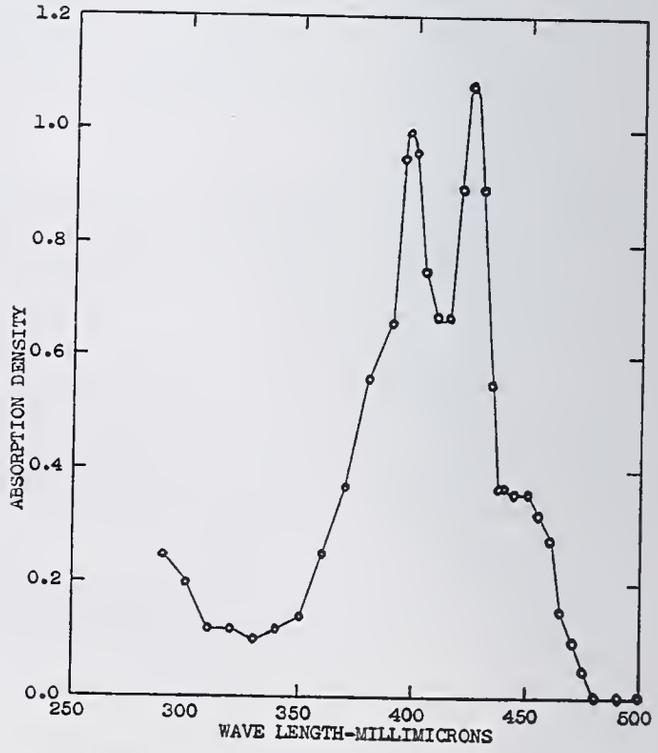

FIGURE 1. ABSORPTION CURVE OF K CAROTENE

Table I. Constituents of Crude Carotene from Yellow Corn

Laboratory No.	Variety	Crude Carotene	Composition of Crude Carotene: Beta-carotene	Alpha-carotene	K carotene	Cryptoxanthin	Neocryptoxanthin	Impurity[a]
		P. p. m.	%	%	%	%	%	%
55,710	Golden June	5.0	30.3	1.4	3.7	50.4	14.1	0.1
55,711	Ferguson Yellow Dent	6.4	30.9	1.7	5.6	44.2	17.5	0.1
55,712	Jarvis Golden Prolific	8.0	26.7	2.5	5.7	47.0	15.5	2.6
55,713	Texas Golden Prolific	6.0	35.6	4.1	7.4	37.7	15.2	0
55,718	Miekle's Yellow Dent	4.0	24.8	0.8	7.9	48.4	18.1	0
54,547	Yellow corn meal	5.3	30.4	5.3	6.5	39.5	18.3	0
54,551	Yellow Surecropper	3.7	28.9	6.1	4.4	41.1	19.4	0.1
54,552	Corn Belt Hybrid, Pfester	1.3	31.0	7.1	3.5	46.1	12.2	0.1
55,847	Johnson's Yellow Dent	6.4	29.9	5.1	5.2	44.5	15.3	0
55,848	Pluger's Mammoth Yellow	6.4	28.3	5.4	5.6	42.4	18.3	0
55,849	Good's Golden Prolific	4.1	33.5	4.2	7.9	41.8	12.6	0
55,719	Reese Yellow Squaw	4.4	32.5	6.1	5.7	40.3	15.4	0
55,720	Yellow Tuxpan	5.0	33.9	1.5	5.3	53.3	5.9	0.1
55,721	Reese Yellow Drought Resister	5.4	29.1	4.5	4.5	36.9	18.5	6.5
55,722	Yellow Paymaster	6.2	34.7	2.9	6.8	38.1	12.6	4.9
55,723	Yellow Surecropper	3.4	23.9	4.0	5.3	38.8	19.4	8.6
55,850	Leudtke's Yellow Sure	4.6	31.6	4.9	5.5	33.3	20.2	4.5
58,941	Yellow Paymaster	4.1	35.8	5.1	6.2	35.5	17.6	0
	Average, Group 1 (18)	5.0	30.7	4.0	5.7	42.2	15.8	1.5
55,714	Golden Thomas	7.3	22.6	3.1	5.6	39.7	29.0	0
55,715	Hastings Golden Dent	7.4	16.8	0.9	6.9	50.0	21.2	4.2
55,716	Hill's Yellow Dent	6.0	18.4	0.2	3.4	53.2	24.7	0.1
55,717	Reese Giant Yellow Dent	3.7	21.8	0	5.2	51.8	21.1	0.1
	Average, Group 2 (4)	6.1	19.9	1.1	5.3	48.7	24.0	1.1
	Average of Groups 1 and 2 (22)	5.2	28.7	3.5	5.6	43.4	17.3	1.5

[a] When less than 1%, impurity is determined by difference.

(*12*) and by spectroscopic examination. The pigment called K carotene was at first thought to be gamma-carotene, but spectroscopic examination showed it to have absorption maxima at 425 and 397 millimicrons. This is very different from gamma-carotene, which, according to Zechmeister (*11*), has maxima in hexane at 494, 462, and 431. The absorption curve of K carotene in petroleum ether is given in Figure 1. It is unlikely that the K carotene was formed from carotene during the chromatographic separation or because of solution. Solutions of a mixture of alpha- and beta-carotene have been chromatographically separated many times in this laboratory and the band of K carotene was never observed to form from them. A survey of the literature indicated that K carotene has not heretofore been reported. It comes nearest to being B-dehydro carotene (*8*) but differs from it in having vitamin A potency.

Zechmeister and Tuzson (*13*) and Carter and Gillam (*3*) have shown that carotinoid pigments isomerize in solution, and according to Zechmeister and Tuzson (*13*) part of the cryptoxanthin in solution easily isomerizes into neocryptoxanthin. Both these groups of workers have shown that the isomerizations are due to solution and not to changes in the column of adsorbent. In order to check these findings, cryptoxanthin was removed from a column, dissolved in petroleum ether, and passed into another column. A band of cryptoxanthin and a band of neocryptoxanthin were always formed. When neocryptoxanthin was eluted and passed into a column, bands of cryptoxanthin and of neocryptoxanthin were formed. Cryptoxanthin and neocryptoxanthin appear to be isomers of each other and in solution either pigment can form the other. Consequently, in the chemical determination of vitamin A potency of yellow corn the neocryptoxanthin may be included with the cryptoxanthin.

The results of the chromatographic analyses for 22 samples of corn are given in Table I, in percentage of total carotinoids recovered from the chromatograph column, so that any loss or gain was prorated. The analyses fall into two groups as regards beta-carotene content and cryptoxanthin. In the last 4 varieties listed, the crude carotene contains 16.8 to 22.6 per cent of beta-carotene with an average of 19.9, while in the group of 18 samples the percentage is 23.9 to 35.8 with an average of 30.7. The cryptoxanthin plus neocryptoxanthin averages 72.7 per cent in the first group and 58.0 per cent in the second. The percentage of alpha-carotene in the crude carotene of the entire group ranges from 0.0 to 7.1 with an average of 3.5 per cent, that of the K carotene from 3.4 to 7.9 with an average of 5.6, that of the cryptoxanthin from 33.3 to 53.3 with an average of 43.4, the neocryptoxanthin from 5.9 to 29.0 with an average of 17.3, and the impurity from 0 to 8.6 with an average of 1.5.

The average pigment lost in the column was about 3 per cent, but in 27 determinations there were three losses of more than 10 per cent, one of which was as high as 18 per cent. In nine of the determinations there was a slight gain of pigment, probably due to error in the colorimetric determinations, and in one determination there was a gain of 14 per cent. When the loss or gain is high, the results should be discarded.

Biological Activity of Pigments

The K carotene, alpha-carotene, cryptoxanthin, and neocryptoxanthin from two samples of yellow corn were tested with rats for vitamin A potency. The extremely fine bands just below the neocryptoxanthin band in the chromatograph were not included with the neocryptoxanthin. The pigments removed when the petroleum ether extracts were washed with 90 per cent methanol, to remove xanthophylls, were also tested.

For the biological tests large amounts of corn were saponified, extracted, and chromatographed in large columns by the technique described above. The pigments were eluted from the magnesium oxide with petroleum ether and ethanol. The petroleum ether was distilled off *in vacuo* and the pigments were taken up in Wesson oil. The amount of pigment in the Wesson oil was estimated as carotene with a photoelectric colorimeter, and the oil was diluted to give the desired concentration. The biological potency of these solutions was determined by the modified U. S. P. method used in the authors' laboratory (*6*).

The results of the biological tests, given in Table II, show that K carotene, neocryptoxanthin, and cryptoxanthin have approximately one half the vitamin A potency of beta-carotene. The xanthophylls which were also tested had no detectable amount of vitamin A potency.

Table II. Vitamin A Potency

(International units per microgram of pigment)

	Corn 57,948	Corn 58,929
Standard carotene in oil	1.7	...
Beta-carotene	1.1	...
K carotene	0.5	0.6
Cryptoxanthin	0.6	0.6
Neocryptoxanthin	0.4	0.4
Xanthophylls	...	0.0

Abridged Chromatographic Method

When separation of all the pigments in the crude carotene extracts of yellow corn is desired, the chromatographic method just described can be used. This method is tedious, and when

Table III. Carotene and Cryptoxanthin by Chromatographic and Abridged Chromatographic Methods

Laboratory No.	Chromatographic Method			Abridged Chromatographic Method		
	Carotene	Cryptoxanthin	Impurity	Carotene	Cryptoxanthin	Impurity
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
58,941	1.9	2.0	0.2	1.7	1.6	0.3
58,942	1.7	1.9	0.1	1.7	1.6	0.3
58,943	0.5	0.4	0.1	0.3	0.3	0.0
58,945	2.6	4.7	0.2	2.8	4.6	0.3
58,946	1.4	2.2	0.1	1.7	2.0	0.3
58,947	0.4	0.5	0.1	0.5	0.5	0.1
58,949	2.6	4.4	0.4	3.0	4.2	0.3
58,950	1.3	2.3	0.1	1.5	2.2	0.1
58,951	0.7	1.5	0.1	0.7	1.3	0.2
58,953	3.7	4.0	0.1	4.4	3.3	0.4
58,954	2.2	3.2	0.1	2.5	2.8	0.2
58,955	1.1	0.8	0.1	1.3	0.7	0.1

analyses are to be run on large numbers of corn samples, a quicker and simpler method is needed. For this purpose an abridged chromatographic method was devised in which only the pure carotene, the cryptoxanthin, and the impurities are determined.

The U. S. P. light magnesium carbonate should be tested by placing approximately 1 gram in a tube as described below and passing a solution of purified carotene (1.0 to 1.5 parts per million) through it, washing with petroleum ether, and determining the carotene in the filtrate. The carotene should be washed through within about half an hour without loss of over 5 per cent. If the magnesium carbonate is too retentive of carotene, another lot should be tried.

An adsorbent column was prepared by placing approximately 1 gram of magnesium carbonate in a glass tube 5 to 8 mm. wide and about 15 cm. tall, constricted at one end and plugged with a wad of cotton. Suction was then applied and the magnesium carbonate was packed firmly but not too tightly with a cork with a smooth surface attached to a glass rod.

For the determination 10 grams of corn meal were saponified, as in the A. O. A. C. method, and the crude carotene extract was diluted to exactly 100 cc. The amount of crude carotene was determined with a photoelectric colorimeter and a 50-cc. aliquot concentrated *in vacuo* to about 15 to 20 cc. A few cubic centimeters of petroleum ether were placed on the column of magnesium carbonate, suction was applied and, before the petroleum ether was drawn in, the crude carotene solution was put in the tube. The magnesium carbonate was then washed with petroleum ether. The pure carotene did not form a band, but passed through the column. This solution was made up to volume and the pure carotene determined. The pure carotene includes alpha-carotene, beta-carotene, and K carotene.

An impurity formed a small band near the bottom of the column, and was washed out after the carotene had been removed. A slight band of impurity was sometimes found at the top of the column. If formed, this was scraped out, eluted with petroleum ether containing 2 per cent of ethanol, and after the ethanol had been washed out with water, it was combined with the impurity from the bottom of the column and diluted to volume and the quantity was determined. The average quantity of impurity is so small that it can be disregarded.

Bands of cryptoxanthin and of neocryptoxanthin were formed above the impurity at the bottom of the tube. They were scraped out together and eluted with petroleum ether and alcohol, the alcohol washed out with water, the solution made up to volume, and the quantity determined.

Samples of corn meal were analyzed by this method and by the complete chromatographic method (Table III). The results by the complete chromatographic method were added to give the corresponding constituents as by the abridged method. Examination of Table III shows that satisfactory agreement was secured, considering the small quantities present.

The method was also tested by putting known amounts of pure carotene through the column. Recovery of 100 per cent was obtained. The recoveries of corn pigments were 95 to 105 per cent.

The principal disadvantages of this method are that alpha-, beta-, and K carotene are not separated and every lot of magnesium carbonate must be tested before it is used.

Calculating Beta-Carotene Equivalent and Vitamin A Potency from Analyses

For some purposes it may be sufficient to calculate the carotene and cryptoxanthin content of yellow corn from the crude carotene. Table I shows that on an average the crude carotene in yellow corn contains 3.5 per cent of alpha-carotene, 28.7 per cent of beta-carotene, 5.6 per cent of K carotene, and 60.7 per cent of cryptoxanthin and neocryptoxanthin combined. Since the alpha-carotene and K carotene have the same vitamin A potency as the cryptoxanthin and only half that of the beta-carotene, they should be included with the cryptoxanthin rather than with the beta-carotene.

The crude carotene is multiplied by 0.29 to get the beta-carotene and by 0.70 to get the sum of the alpha-carotene, K carotene, and cryptoxanthin.

Carotene in feeds is usually expressed as beta-carotene. If the equivalent in beta-carotene is desired, the crude carotene is multiplied by 0.64 ($0.70 \times 0.5 + 0.29$). If the vitamin A potency in U. S. P. units is desired, the crude carotene is multiplied by 1.1 (0.64 divided by 0.6).

Although this method is only approximate, the results are probably more accurate than those of a biological assay. The figure 1.1, which is the number of International Units of vitamin A potency per microgram of crude carotene in yellow corn, agrees fairly well with 1.4, the figure found by Fraps, Treichler, and Kemmerer (7). The beta-carotene equivalent of the 22 samples in Table I was calculated both from the crude carotene only and from the results of the chromatographic analyses in Table I. The average difference of the 22 samples was 0.15 part per million, and the standard deviation was 0.06. If the variety of corn is known, the calculation should be more exact if the average composition of the group to which this variety belongs is used (Table I) instead of the average of all the samples of corn.

Summary

The alpha-carotene, beta-carotene, K carotene, cryptoxanthin, neocryptoxanthin, and impurity in yellow corn can be determined by a chromatographic method. An abridged method may be used for determining the carotenes, cryptoxanthin, and impurities. The approximate quantities of these constituents can be calculated from the crude carotene and the beta-carotene equivalent and U. S. P. vitamin units can be calculated from the crude carotene with a fair degree of accuracy.

Twenty-two samples of yellow corn differing in genetic strain were analyzed by the chromatograph method. Two groups of corn were observed, one containing on an average 19.9 per cent beta-carotene and 72.7 per cent cryptoxanthin in the crude carotene, the other and larger group containing 30.7 per cent beta-carotene and 58.0 per cent cryptoxanthin in the crude carotene. The average percentages found were beta-carotene 28.7, alpha-carotene 3.5, K carotene 5.6, cryptoxanthin 43.4, and neocryptoxanthin 17.3. Neocryptoxanthin was formed when cryptoxanthin was dissolved, and cryptoxanthin was formed when neocryptoxanthin was dissolved, so that cryptoxanthin may be considered to be the sum of the two.

A new form of carotene, termed K carotene, is found in yellow corn. It has a biological potency equal to that of alpha-carotene.

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Fluorescent Tests for Beryllium and Thorium

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FLUORESCENT tests for both beryllium and thorium with morin and cochineal have been described by Goto (*1*), but in neither case is the reaction specific, since these reagents produce a fluorescence with many other cations. Recently morin as a reagent for beryllium has been thoroughly studied by Sandell (*2*). The response of beryllium and thorium to a common reagent is also shown by 1,2,5,8-tetrahydroxyanthraquinone (quinalizarin), which gives the well-known cornflower blue color with beryllium. Willard and Winter (*3*) have noted that thorium also gives a characteristic color reaction with this material.

In a search for reagents to give specific fluorescent tests with the cations, the idea of testing the hydroxyanthraquinones for beryllium and thorium presented itself. Several of these were tried and it was found that 1-amino-4-hydroxyanthraquinone gives an intense fluorescence with beryllium in alkaline solution and with thorium in acid solution.

Characteristics of Reagent

The reagent, 1-amino-4-hydroxyanthraquinone, is a purplish-red powder, insoluble in water but soluble in alcohol. Water solution of alkalies, acids of concentration greater than 0.5 *N*, and alcohol solutions of over 50 per cent dissolve the compound. The color in alcohol and acids is red and in bases is purple. The alcohol and acid solutions fluoresce red, but the alkaline solutions do not fluoresce. If an alcohol solution of the material is added to a slightly acid solution of almost any cation other than thorium, a curious mode of precipitation takes place. A sacklike membrane seems to form on the walls of the test tube. This gradually contracts and the film of the same shape as the tube becomes smaller and smaller until it forms a flocculent suspension on the surface of the solution. Th^{++++} is the only ion that will keep this high-colored reagent dispersed in dilute acid solution. Oxidizing agents destroy the reagent; however, it seems to be stable in the presence of air. No changes in alcohol solutions are noticed after a period of several months.

For testing purposes an approximately 0.1 per cent solution was made by dissolving 0.1 gram of the reagent in 100 ml. of 95 per cent ethyl alcohol. It is sometimes necessary to warm the alcohol for about 15 minutes to effect complete solution.

Apparatus

The source of the ultraviolet rays used in these experiments was the 100-watt, type 4, red-purple bulb, mercury lamp of the General Electric Vapor Lamp Company, Hoboken, N. J., which gives radiations between 3100 and 4000 Å. with a maximum at 3650 Å. Observations were made in a partially darkened room and ordinary soft-glass test tubes were used as containers.

Beryllium Test

The test solutions used contained 0.1 gram of beryllium per liter. In making the test 0.1 ml. of this was placed in a test tube and 1 ml. of 2.5 *N* sodium hydroxide was added. This was diluted to 10 ml. and 0.5 ml. of 0.1 per cent alcohol solution of 1-amino-4-hydroxyanthraquinone was added. This solution under the ultraviolet lamp gave a red fluorescence extending from about 6300 to 6800 Å. In visible light the beryllium solution had the same purple color as an alkaline solution of the reagent. The test is excellent to the extent of one part of beryllium in 10^6 parts of water and can be observed in a dilution of 1 in 4×10^7 parts. One or 2 ml. of solution can be easily observed, but it is better to use over 5 if that quantity is available. At this concentration the test is much more definite than the familiar one with quinalizarin.

If such a quantity of beryllium salt is used that the hydroxide does not dissolve in 1 ml. of 2.5 *N* sodium hydroxide, a 10 per cent solution of sodium potassium tartrate is added drop by drop until the precipitate dissolves. An excess of sodium hydroxide must be avoided, since over 0.3 *N* alkali causes an appreciable diminution in the fluorescence. Mixtures containing beryllium were run through the usual procedure of analysis and the beryllium was easily detected in the presence of the aluminum by dissolving the hydroxide precipitate in sodium hydroxide and adding the reagent.

Interfering Ions. Reasonable quantities of cations and anions, except lithium, have no effect on this test. It requires 20 grams of sodium chloride in 100 ml. of solution to destroy the fluorescence. Lithium in concentrations of 0.007 gram per 10 ml. or greater gives a fluorescence like that of beryllium. This similarity in action of lithium and beryllium was also noted by Sandell (*2*) when using morin as a reagent. Colored ions such as Cr^{+++} in small quantities cause no difficulty, but a deep green solution will mask the fluorescent color. The other cations which remain unprecipitated in 0.2 *N* alkali seem to have no effect. Calcium ions in saturated calcium hydroxide produce a faint fluorescence, but the amount remaining after addition of 0.2 *N* sodium hydroxide has no effect. Small amounts of iron causing a brownish color need not be precipitated but may be rendered noninterfering by the addition of tartrate. The cations listed below under thorium were examined in this test.

The common anions, such as chloride, nitrate, sulfate, borate, and fluoride, have no effect. Ions which precipitate beryllium in alkaline solution, such as phosphate, arsenate, molybdate, tungstate, and uranate, may be nullified by having tartrate present. Tartrate decreases slightly the intensity of the beryllium fluorescence, but this is not serious unless exceedingly large concentrations of tartrate are used with small concentrations of beryllium. It requires 0.5 gram of sodium potassium tartrate tetrahydrate to destroy the fluorescence of 1 microgram of beryllium. Chromate oxidizes the reagent and destroys the test. If ammonium ions are present in the solution, care must be taken to make the solution decidedly

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alkaline, since these ions will neutralize an equal quantity of sodium hydroxide.

Thorium Test

Thorium salts in weakly acid solutions with 1-amino-4-hydroxyanthraquinone produce a purple colloid which gives a red fluorescence of the same wave-length range as that given by the beryllium in alkaline solution. With this reagent thorium gives a fluorescence only in acid solution and beryllium only in alkaline solution.

The thorium solution to be tested must be adjusted to a pH of about 2 without too large a quantity of salt present. This is best accomplished by using thymol blue as an indicator and adding acid or alkali until the yellow point representing a pH of 1.75 is reached. Neutralizing to the phenolphthalein end point precipitates the thorium, which is rather difficult to redissolve. In addition, the quantity of salt produced in this operation is likely to precipitate the thorium colloid formed. It is important not to have the solution too acid, since the reagent itself dissolves to a sufficient extent in 0.5 *N* acid to give an intense fluorescence. The purple color of the thorium complex in visible light is given in the acid solution by no other element except zirconium, and it is only to distinguish this that the fluorescence need be used. The test is not apparent for less than 40 micrograms of thorium in a dilution of 1 to 125,000.

Thorium nitrate or chloride forms the most convenient test solution, which is made to contain 1 gram of thorium ion per liter. The acidity of this concentration is satisfactory for the test without further adjustment. One milliliter of this solution is mixed with 10 ml. of water and 0.5 ml. of 0.1 per cent 1-amino-4-hydroxy anthraquinone is added. The mixture is examined either directly or under the ultraviolet lamp.

In testing for thorium from highly acidic mixtures such as are obtained from the separation of monazite sand, it is necessary to precipitate the thorium as a hydroxide, iodate, or oxalate, filter, and redissolve in concentrated hydrochloric acid or aqua regia. The latter is used in the case of the iodate and oxalate and hydrochloric acid is added to this until all the nitrate is removed. The material is then evaporated just to dryness to remove the excess hydrochloric acid, and the crystals are dissolved in water and adjusted to the proper pH with thymol blue.

Interfering Ions. The only cations, other than thorium, found to give the slightest fluorescence under the above conditions were gallium and praseodymium, which give a weaker fluorescence of a little darker shade than that of thorium. If a trace of thorium is mixed with these elements, the intensity is much greater and there seems to be little chance of confusion. Both gallium and praseodymium produce red solutions in contrast to the purple of thorium and, on standing a few minutes, they form red curdy precipitates, whereas the thorium is permanently stable. It requires 1.5 mg. of gallium and 10.0 mg. of praseodymium to produce the same fluorescence as 0.1 mg. of thorium. Zirconium and ferric ions do not cause a fluorescence but decrease the intensity of thorium. Oxidizing agents of the order of Ce^{++++}, Ag^{+}, Au^{+++}, Hg^{+}, and the ions of the platinum metals destroy the reagent.

In addition to those already indicated, solutions of the following cations were examined and found to have no effect: lithium, sodium, potassium, rubidium, cesium, copper, beryllium, magnesium, calcium, barium, strontium, zinc, cadmium, aluminum, lanthanum, cerous cerium, neodymium, a mixture of the rare earths as taken from monazite, indium, thallium, zirconium, hafnium, tin, lead, bismuth, antimony, chromium, manganese, cobalt, and nickel. The influence of some of the anions seems to be the effect of charged ions on a sensitive colloid, and in other cases precipitation of the thorium takes place. It required 4 grams of sodium chloride in 10 ml. of solution to destroy the colloid, and hence the fluorescence, produced by 2×10^{-3} gram of thorium. Phosphates, fluorides, and sulfates present in quantities expressed in grams per liter half as large as the thorium destroy the fluorescence. Iodates, arsenates, oxalates, molybdates, tungstates, and uranates precipitate the thorium.

Results with Other Hydroxyanthraquinones

With 1,8-dihydroxyanthraquinone, beryllium in alkaline solution gives a red fluorescence which is not so intense as that described above but can be used to detect beryllium in dilutions as low as 1 in 10^6. The smallest quantity detected was 1×10^{-5} gram. The other ions soluble in sodium hydroxide do not interfere. Neither beryllium nor thorium fluoresces in acid solution with this reagent.

In alkaline solution 1,5-dihydroxyanthraquinone gives a slight fluorescence with beryllium but is not of sufficient intensity to serve as a qualitative test. Neither beryllium nor thorium affects this reagent in acid solution.

No fluorescence is given by 1,2,5,8-tetrahydroxyanthraquinone with either beryllium or thorium. In slightly acid solution this reagent with aluminum gives a beautiful orange-red fluorescence (6100 to 6500 Å.) which is destroyed by the addition of thorium; 2×10^{-5} gram of thorium will destroy the fluorescence of 1×10^{-3} gram of aluminum in 10 ml. of solution. The reagent is sensitive to only 1×10^{-4} gram of aluminum in 10 ml. and this is destroyed by many other ions and by addition of acid; hence it cannot be considered a good test reagent for either aluminum or thorium.

4,8-Diamino-1,5-dihydroxy-2-sulfonic acid anthraquinone does not fluoresce with either thorium or beryllium; 1-amino-5-hydroxyanthraquinone gives a slight fluorescence with beryllium in alkaline solution but none with thorium in acid solution.

Summary

The reagent 1-amino-4-hydroxyanthraquinone serves well for detecting beryllium in alkaline solutions and thorium in acid solution. In the case of beryllium, the test is less sensitive but more specific than morin, and is much more definite than with quinalizarin.

In application to thorium the sensitivity is not so great as might be desired, but is sufficient for many practical purposes and provides a vivid color reaction for the identification of this element. Several hydroxyanthraquinones were tested with these elements and the only other one found to present analytical possibilities was 1,8-dihydroxyanthraquinone.

While all possibilities have not been tried, it seems obvious that tests with metallic ions may assist in identifying the location of groups in the anthraquinones.

Acknowledgment

The authors wish to express their appreciation to W. Reeve and W. H. Power of the Organic Department of the university for preparing the 1-amino-4-hydroxyanthraquinone and to J. Lander for preparing the 1-amino-5-hydroxyanthraquinone.

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Correction. In the article entitled "A Fluorescent Method for Aluminum" [Ind. Eng. Chem., Anal. Ed., 9, 430 (1937)], we find it an improvement to make up the Pontachrome Blue Black R in 95 per cent ethyl alcohol and to heat the test solution to about 80° C. before adding this reagent. This gives a permanently stable reagent, and using the higher temperature gives an immediate result with low concentrations of aluminum which would require several hours to develop at room temperature.

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A Continuous Calcium Carbonate Saturation Balance Indicator

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INTERNAL protection of most pipe lines carrying water supplies depends mainly upon the formation and maintenance of a thin protective scale high in calcium carbonate. The tendency of a water either to corrode metal or to lay a protective film may be determined experimentally by the magnitude and direction of its changes in alkalinity and pH when contacted with calcium carbonate, employing the so-called marble test (*3*). Until Enslow (*1*) devised a continuous flow tube packed with calcium carbonate, experimental technique consisted in mechanically agitating batch samples in various types of containers with calcium carbonate powder.

The assembly described below is an amplification of the Enslow tube with additional provisions which include (1) sufficient length of packed column to ensure equilibrium for the water undergoing test, (2) an internal filter to prevent powder carry-over, (3) a companion unpacked column, or synchronizing tube, to preserve proper time relationship between the original water and its stabilized counterpart, especially applicable for rapidly changing supplies, and (4) continuous e. m. f. indication by means of an integral concentration cell. As a safeguard against gas release from supplies high in carbon dioxide, water is made to flow within the apparatus against a positive head. Effects of momentary aeration, ordinarily permitted when using external electrodes, are completely avoided by making measurements within the closed path of water travel. With suitable arrangements for heating and insulation, measurements may be made at elevated temperatures. As a single operating requirement, this apparatus (Figure 1) uses a large volume of relatively clear water under pressure.

The logical locales in which this device is expected to serve most effectively in guiding plant operation to maintain effluents near calcium carbonate balance are:

Plants employing simple anticorrosion treatment, such as aeration or limestone beds. Mildly corrosive effluents are known to diminish pipe-line carrying capacity, even though red water is not evident; hence the value of a sensitive automatic indicator for this type of supply.

Plants controlling corrosion by means of alkali. The same point applies as above, with the added requirement that the treatment be not overstepped; otherwise excessive scaling is induced in hot-water fixtures.

Softening plants employing recarbonation. Proper control of the extent of recarbonation is essential, as the calcium carbonate balance for softened waters of this type may be sensitive.

Zeolite installations having a low alkalinity in the effluent. With a water of this character, which is deprived of the protective effect of both calcium and bicarbonate ions, a continuous indicator may be helpful in controlling blends with a small proportion of raw water.

For untreated aggressively corrosive waters the use of this apparatus is unnecessary, as the appearance of the effluent is sufficient evidence of corrosive attack on pipe-line metal.

Description and Operating Notes

Influent under pressure is divided equally, by means of petcocks *G* and *H* at the bottom tee connection, between the powdered calcium carbonate column, *C*, and the empty synchronizing tube, *B*, whose volume is made to equal the void space in *C*. Tube *B* thus provides a detention period equal to that of column *C* and in this way furnishes a continuous reference sample of the original water for comparison with its corresponding stabilized effluent from *C*. When leaving *C*, effluent in equilibrium with the calcium carbonate solid phase (equilibrate) passes through a coarse sintered-glass crucible or filtering tube which prevents carry-over. The packing in *C* rests on a layer of pea gravel sandwiched between retaining pads of glass wool. A vent is provided at the top for releasing air that accumulates above the crucible level when *C* is first placed in service.

Effluents from *B* and *C* flow through Gooch crucible holders *N* and *M*, respectively, which contain an antimony or glass electrode pair to form a simple concentration cell that connects with a vacuum tube potentiometer, preferably of the recording type. *X*, a potassium chloride–agar bridge, completes the circuit in which liquid junction errors cancel by symmetry. No calomel reference electrode is needed, as the differential in pH is the quantity to be measured and its value is read directly from the potentiometer. Cell effluents flow to waste continuously at the high points, W_E and W_T, after first filling the 250-ml. Erlenmeyer flasks, S_E and S_T, to furnish a continually available set of current samples for alkalinity titration.

The author's calcium carbonate column, measuring 4.8 cm. (1.875 inches) in diameter by 112 cm. (44 inches) high, allows adequate contact for the slightly scaling local water. A flow rate of from 4 to 6 ml. per minute is satisfactory; faster rates cause excessive back pressure at the bottom of column *C*, while slower rates permit an extended time lag and so may fail to represent prevailing plant conditions. The friction head ordinarily is about 137 cm. (4.5 feet) of water, although this quantity varies with the rate of flow and the degree of compaction of the powder. At the bottom of *C* the rubber stopper must be strapped securely with

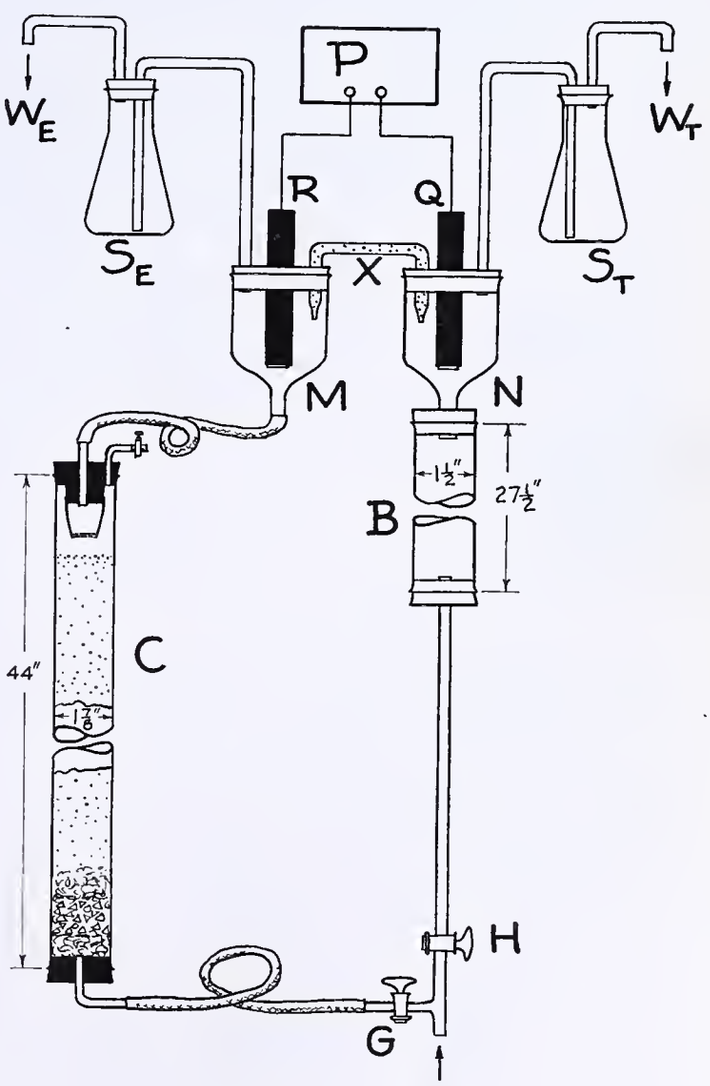

FIGURE 1. ASSEMBLY

adhesive tape to prevent a blowout. In order to expand the powder and thereby reduce the loss of head, direction of flow is upward through the column. During operation the powder settles to a level several centimeters below the crucible, so that generally only slightly turbid equilibrate reaches the filter, as the packing acts as its own upflow filter; the powder also separates at times somewhere near the middle of the column by a water space of variable length between the lower and upper portions. Through this lower portion the ascending water tends to channel against the smooth glass wall but, beyond the central liquid partition level, penetrates upward through the remainder of the packing by percolation. A number of deflecting devices placed in the bottom of *C* failed to prevent channeling and were abandoned.

It is evident that short tubes fail to guarantee complete equilibrium because of both insufficient length and lack of intimate contact. Connections to *C* are made with liberal lengths of rubber tubing to allow shaking and overturning every few weeks to break up lumps and reduce the loss of head through the powder. Gravel bottoms were found superior to simple glass wool layers in preventing powder from falling through the bottom of *C* into the inlet connection. Glass wool padding was inserted as indicated to keep the powder and the gravel in place during the overturning action.

Coated filter sand and two varieties of crushed marble were tried as packing material but with lime-softened water caused too low a phenolphthalein reading in the effluent, apparently converting some of the normal carbonates into bicarbonate alkalinity. Precipitated calcium carbonate, C. P. grade, proved to be the most reliable contact medium after the assembly was aged to leach out soluble impurities. For the internal filter, a Berkefeld candle, porosity N, was tried for a month, but did not age completely and an Alundum crucible gave indifferent results. However, no type of filter showed any tendency to clog when filtering equilibrated water.

Electrodes were checked occasionally by comparing their e. m. f. in the same solution. This was done externally rather than by adding a by-pass between vessels *M* and *N*. The pH value of the tap sample was conveniently determined in cell *N* by inserting a calomel electrode and placing electrode *Q* in another potentiometer circuit with it.

Table I. Calcium Carbonate Stability of New Orleans Tap Water

(Preliminary data)

	6/22/39	7/13/39	11/21/39
Tap water			
pH	9.88	9.79	9.81
Alkalinity, p. p. m. $CaCO_3$			
Phenolphthalein	17	15	27
Methyl orange	33	30	53.5
$CaCO_3$ column equilibrate			
Δ pH	−0.29	−0.30	−0.12
pH	9.59	9.49	9.69
Alkalinity, p. p. m. $CaCO_3$			
Phenolphthalein	10	7	18
Methyl orange	21.5	15	38
Bottle test supernatant			
pH	9.61	9.55	....
Alkalinity, p. p. m. $CaCO_3$			
Phenolphthalein	10	11	....
Methyl orange	19	22	....

Application

So far this apparatus has been used only with New Orleans tap water, a lime-softened supply from the Mississippi River, toward which the column functions as a desupersaturator. In view of this limited experience, these results and observations are offered as a preliminary study. While there was no opportunity to try the assembly on an aggressive water, it is believed that the column should work qualitatively, at least, since ordinary bottle test technique for quick results on corrosive waters requires only a 5- to 10-minute contact. Operating data were obtained over a period of 6 months. For comparison an occasional bottle test was run in parallel by turning a tap sample, held in a special cage, overnight during off hours in the machine-shop lathe. This agitation in contact with C. P. calcium carbonate was followed by 8 hours' settling before pipetting off a portion of the supernatant for titration. Data typical of results obtained are given in Table I.

Almost without a single exception, more complete stabilization was obtained in the column than in bottle tests, even though bottle agitation was extended to 60 hours, as evidenced by lower pH values and alkalinities by the column method. Throughout the period of use alkalinity values in the column effluent approached the theoretical solubility for calcium carbonate, indicating equilibrium in this regard. However, passage through the calcium carbonate tower usually caused a loss of about 1 part per million of phenolphthalein alkalinity beyond that reduced by stabilization, this result causing an apparent gain in bicarbonates ranging from 0 to 3 p. p. m.

In searching for the cause for this slight drop in the phenolphthalein alkalinity of the equilibrate, other properties than calcium carbonate quantities were determined and some were found to change on percolation through the column. Chlorine residuals of about 0.7 p. p. m. in the tap, due to chloramines, disappeared entirely in the equilibrate; instead of the usual light green residual a strong nitrite interference color, characteristically brown, formed slowly with *o*-tolidine. Ordinary bottle tests, on the contrary, always retained a strong chlorine residual. Production of nitrites within the packing from free ammonia and that added for chloramine formation was evident, and it was found that nitrite increased from 0.10 p. p. m. in the tap to 1.68 p. p. m. in the column effluent, whereas the bottle test supernatant was raised only to 0.115 p. p. m. Presence of Nitrosomonas, suggested by the elevation in nitrites, was demonstrated in the packing (*2*), although both tap water and column effluent were sterile on A. P. H. A. nutrient agar. These effects, by releasing small amounts of nitrous and hydrochloric acids which transformed normal carbonates into bicarbonates, were probably responsible for the slight depression in phenolphthalein alkalinity and in pH of the stabilized effluent from the apparatus.

Nitrate nitrogen increased very slightly from 0.10 p. p. m. in the tap to 0.20 p. p. m. in the tube effluent but the nitrate content in the bottle test remained unaffected. Passage through the column caused a drop of 1 p. p. m. in dissolved oxygen but oxygen consumed from potassium permanganate showed practically no change.

Gravimetric calcium was determined in the column effluent on several occasions in order to calculate pH_s in Langelier's formula (*4*). The calculated value for pH_s was from 0.3 to 0.6 pH unit less than the pH value of the equilibrated effluent.

Compared with the bottle test this column is decidedly superior, as it destroys supersaturation usually from 2 to 7 p. p. m. of calcium carbonate more completely and at the same time provides automatic, continuous, and reasonably current information on plant production. In waters containing free ammonia or chloramines the equilibrium pH value is slightly depressed by nitrite formation and hydrochloric acid release, but the small error of less than 0.05 pH lies on the side of safety in the case of the local water and the differential obtained with this assembly is an index of the tendency to deposit scale.

Acknowledgment

Most of the experimental work was performed in the New Orleans Water Purification Plant Laboratory. All glass electrodes and a Coleman potentiometer were borrowed from sources in the vicinity.

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Separation and Determination of Lead with Salicylaldoxime

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SALICYLALDOXIME has been used as a reagent for the gravimetric determination of copper (*3*), nickel (*9*), palladium (*5*), lead (*6*), bismuth (*4*), and zinc (*4*). Separations of copper from nickel (*1, 2, 9*), copper from lead (*7*), bismuth from zinc (*4*), and bismuth from silver (*4*) have been carried out by control of pH or the use of ammonia complex formation. A careful study has been made of the effect of pH on the precipitation of copper salicylaldoximate and nickel salicylaldoximate (*1*). It seemed desirable to study lead in a similar manner, inasmuch as the data obtained could be used as the basis for separating lead from other metals.

Determination of Optimum pH for Precipitation

SOLUTIONS. Standard lead nitrate solutions were prepared from twice-recrystallized reagent grade lead nitrate, dissolved in water containing approximately 0.02 mole of redistilled nitric acid per liter. Standard lead acetate solutions were prepared from twice-recrystallized analytical reagent lead acetate dissolved in 0.02 molar acetic acid solution. The lead solutions were standardized by precipitation of the lead as lead sulfate.

The salicylaldoxime was obtained from the Eastman Kodak Company. A 1 per cent solution was prepared according to Ephraim (*3*) by slowly pouring a solution of 1 gram of salicylaldoxime in 5 ml. of 95 per cent ethyl alcohol into 95 ml. of water heated to 80° C. The solution was cooled and filtered before using. It was freshly prepared the same day as used.

PRECIPITATION TECHNIQUE. A 10-ml. portion of a 1 per cent salicylaldoxime solution was added to 25-ml. portions of the standard lead solutions. Ammonia solution was added in amounts estimated to give pH values over the desired range, and in each case water was added to give a total volume of 65 ml. The resulting precipitates and solutions were each stirred for 1 hour, and the precipitates were washed by decantation and filtered with suction through No. 4 Jena glass filtering crucibles. The precipitates were washed with water until free from salicylaldoxime as shown by the absence of coloration of the filtrate upon addition of ferric chloride solution, dried at 105° C. for 1 hour, and weighed as $PbC_7H_5O_2N$.

MEASUREMENT OF pH. The hydrogen-ion concentration was measured with a glass electrode pH meter (*8*) calibrated with Clark and Lubs buffer solutions. The measurement was taken upon the first filtrate decanted from the precipitate, and before any washings had been added. The pH values were reproducible to ±0.02 pH unit.

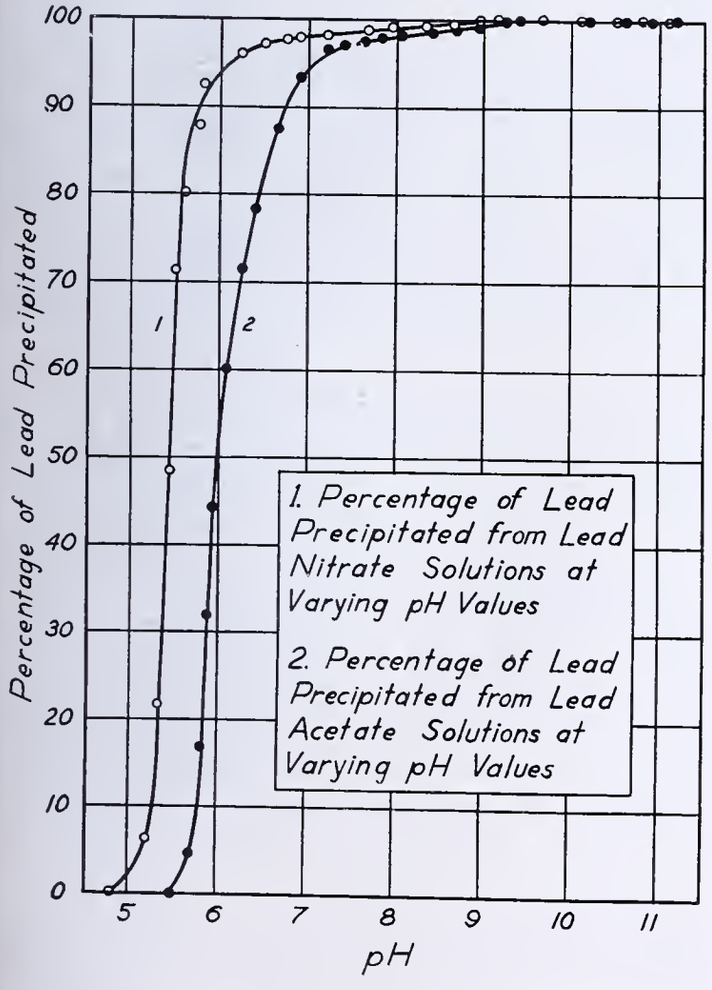

FIGURE 1. PRECIPITATION OF LEAD WITH SALICYLALDOXIME

pH RANGE FOR PRECIPITATION. The results of the precipitation of lead from lead acetate solutions are shown by curve 2, Figure 1. The amount of lead present in each determination was 0.1095 gram. The weight of precipitate should be 0.1809 gram if precipitation was complete and if the formula $PbC_7H_5O_2N$ (Pb = 0.6053) was applicable to the compound precipitated. The results show that precipitation begins at about pH 5.5, but that the pH must be equal to or greater than 9.4 before lead can be determined as lead salicylaldoximate using the theoretical factor 0.6053. Precipitation of the lead was actually complete at a much lower pH than 9.4, as shown by the saturation of the filtrate with hydrogen sulfide. There was no evidence of formation of lead sulfide in any of the filtrates with pH above 7.3, indicating that the precipitates formed up to pH 9.4 are a mixture of lead salicylaldoximate and a basic lead salt. This is contrary to previously published results (*6*), which state that it is only necessary to have the pH above 6.5 to obtain complete precipitation of lead as $PbC_7H_5O_2N$.

Curve 1, Figure 1, is a plot of representative data obtained using lead nitrate solutions. The amount of lead present in each determination was 0.0971 gram, which should give a precipitate weighing 0.1604 gram on the basis of complete precipitation as $PbC_7H_5O_2N$. As shown by the curve, precipitation of lead salicylaldoximate begins just above pH 4.8 and is complete as $PbC_7H_5O_2N$ above pH 8.9. Precipitation of lead was actually complete at pH 6.9 and above, as evidenced by no precipitation upon saturation of the filtrate with hydrogen sulfide, but the theoretical factor 0.6053 could not be applied to the precipitate obtained in the pH range of 6.9 to 8.9.

The effect of the acetate ion, even though present in relatively small concentration, is to shift the curve above 0.5 pH unit to the right. Higher acetate-ion concentration would probably necessitate an even higher pH to obtain complete precipitation. It has been reported (*4*) that complete precipitation of lead can be prevented by use of ammonium acetate.

The curves become nearly horizontal during precipitation of the last few percentages of lead. This flat portion of the curve, extending over about 2 pH units, represents the interval during which precipitation of the lead is complete, not solely as lead salicylaldoximate, but probably as a mixture of the salicylaldoxime complex and a basic acetate or nitrate. Thus, if it is desired only to separate the lead from solution, a pH of 6.9 for the nitrate solution and 7.3 for the acetate solution will suffice; but to weigh the precipitate as $PbC_7H_5O_2N$, the pH must be above 8.9 for the nitrate solution, and above 9.3 for the acetate solution.

Table I. Ammonia Required to Dissolve Salicylaldoximates

Metal Salicylaldoxime	Volume of Concentrated NH_3 Added	NH_3 Concentration of Resulting Solution
	Ml.	*Molar*
Silver	0.05	0.01
Zinc	0.4	0.1
Cadmium	1.2	0.3
Nickel	6.6	1.5
Cobalt	6.7	1.5
Copper	50.0	7.0

Separation of Lead

These data show that lead can be quantitatively precipitated from strongly ammoniacal solution. It seemed likely that the separation of lead from other metals which form insoluble complexes with salicylaldoxime might be effected if precipitations were carried out in solutions containing a high concentration of ammonia.

Solubility of Complexes in Ammonia. Solutions of Bi^{+++}, Cd^{++}, Co^{++}, Cu^{++}, Fe^{++}, Mg^{++}, Mn^{++}, Hg^{++}, Ni^{++}, Ag^{+}, and Zn^{++} ions were prepared from reagent grade salts to contain 4.0 grams of the metal per liter of solution. To 10 ml. of each of these solutions were added 20 ml. of a 1 per cent salicylaldoxime solution. The solutions were diluted to 65 ml. with water. One drop of 4 molar ammonia solution was added to each to produce precipitation of the metal salicylaldoximate, except that the addition of ammonia was not necessary to produce precipitation with copper or cobalt. Concentrated ammonia solution was then added dropwise, with stirring, to determine which of the complexes were soluble in ammonia solution, and the relative ease with which they dissolved.

It was found that bismuth, ferrous, magnesium, manganous, and mercuric salicylaldoximates did not dissolve in ammonia solution in concentrations up to 8 molar. The salicylaldoximates of the metals which form ammonia complexes dissolved upon addition of varying amounts of ammonia, as shown in Table I. These results do not represent equilibrium conditions; the precipitates undoubtedly would have dissolved with less ammonia if a longer time had been allowed between addition of successive increments. However, from an analytical standpoint, the data as given are more valuable than would be the data for equilibrium conditions.

Separation Technique. Solutions of copper, cobalt, zinc, and cadmium were prepared by dissolving reagent grade acetate salts in 0.02 *M* acetic acid. Nickel and silver solutions were prepared from reagent grade nitrates dissolved in 0.02 *M* nitric acid. Each solution contained 4.0 grams of metal per liter.

Fifteen-milliliter portions of 1 per cent salicylaldoxime solution were added to mixtures of lead acetate solutions, prepared and standardized as described above, and solutions of the various metals from which precipitation was to be attempted. Concentrated ammonia solution was then added in amount sufficient to precipitate lead salicylaldoximate and to prevent precipitation, if possible, of the salicylaldoximate of the other metal. Water was added to make 65 ml. The precipitate and solution were stirred for 1 hour, and the precipitate was then permitted to settle. The supernatant liquid was decanted through a No. 4 Jena glass filtering crucible. The precipitate was washed, by decantation, with a solution of salicylaldoxime and ammonia of the same concentration as that in which precipitation had occurred. Finally, the precipitate was washed with 20 per cent ethyl alcohol solution, by decantation and also after transfer to the filtering crucible, until it was free from salicylaldoxime. It was dried for 1 hour at 105° C., cooled, and weighed as $PbC_7H_5O_2N$.

Copper. Attempts to separate lead from various mixtures of copper solution and lead acetate solution were unsuccessful. Copper salicylaldoximate precipitated with lead salicylaldoximate from solutions with ammonia concentrations as high as 8 *M*.

Cobalt. Portions of the cobalt solution were mixed with lead acetate solution and separations were attempted employing the technique outlined above. The data in Table I indicate that cobalt salicylaldoximate would not precipitate in 1.5 molar ammonia solution, but coprecipitation of the cobalt complex with the lead actually occurred in ammonia concentrations up to 8 *M*. The precipitate obtained in all cases was a mixture of cobalt and lead salicylaldoximates.

Nickel. The results obtained were very similar to those with cobalt. Coprecipitation of nickel salicylaldoximate was not so pronounced as was the coprecipitation of cobalt salicylaldoximate, but was sufficient to make separations impossible.

Silver. Separations of lead from mixtures containing portions of the silver solution and of the lead acetate solution were accomplished, following the procedure given above. The results are listed in Table II. A volume of 2.5 ml. of concentrated ammonia solution was used in a total volume of 65 ml. of mixture. The data in Table I suggest that a much lower concentration of ammonia would prevent precipitation of silver salicylaldoximate.

Zinc. It was possible to precipitate lead salicylaldoximate uncontaminated by the zinc complex from mixtures of the zinc solution and lead acetate solution (Table II). Although zinc salicylaldoximate does not precipitate from a zinc solution containing less than 0.5 ml. of concentrated ammonia solution in a total volume of 65 ml., it was necessary to have 12.5 ml. of concentrated ammonia solution in a total volume of 65 ml. to prevent coprecipitation of zinc salicylaldoximate with lead salicylaldoximate.

Cadmium. The experiments on the separation of lead from cadmium gave results similar to those with zinc (Table II). A volume of 12.5 ml. of concentrated ammonia solution was used in a total volume of 65 ml.

Metals Not Forming Ammonia Complexes. Since the solubility experiments showed that bismuth, ferrous, manganous, magnesium, and mercuric salicylaldoximates do not appreciably dissolve in ammonia solutions, separations of lead from these ions by salicylaldoxime and high ammonia concentrations are impossible.

Table II. Determination of Lead

Lead Present	Metal Present	Precipitate Found	Lead Found	Error
Gram	*Gram*	*Gram*	*Gram*	*Mg.*
		In the Presence of Silver		
0.0219	0.100	0.0363	0.0220	+0.1
0.0438	0.080	0.0722	0.0437	−0.1
0.0876	0.080	0.1447	0.0876	0.0
0.1095	0.060	0.1808	0.1094	−0.1
		In the Presence of Zinc		
0.0438	0.100	0.0725	0.0439	+0.1
0.0876	0.080	0.1447	0.0876	0.0
0.0876	0.080	0.1450	0.0878	+0.2
0.1095	0.060	0.1811	0.1096	+0.1
		In the Presence of Cadmium		
0.0219	0.100	0.0362	0.0219	0.0
0.0438	0.100	0.0725	0.0439	+0.1
0.0657	0.100	0.1082	0.0655	−0.2
0.1095	0.060	0.1810	0.1096	+0.1

Summary

Lead can be determined by precipitation as lead salicylaldoximate, weighing the resulting precipitate after drying at 105° C., and calculating the amount of lead on the basis of the formula $PbC_7H_5O_2N$. From nitrate solutions, precipitation begins just above pH 4.8 and is complete at 6.9. However, the precipitate obtained at pH 6.9 does not have the composition represented by the formula $PbC_7H_5O_2N$. Precipitation must be carried out at pH 8.9 or above in order to obtain a compound to which the theoretical factor of 0.6053 for lead in lead salicylaldoximate can be applied. The effect of acetate concentrations as low as 0.05 molar is to increase the pH necessary for the beginning of precipitation, for complete precipitation, and for complete precipitation as lead salicylaldoximate. This increase is about 0.5 pH unit.

Lead can be separated as the salicylaldoximate from silver, cadmium, and zinc in strongly ammoniacal solutions.

Salicylaldoxime reagent cannot be used in conjunction with high ammonia concentrations to separate lead from one or more of the following metals in solution: copper, nickel, cobalt, bismuth, iron, magnesium, manganese, and mercury.

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Determination of Starch by the A. O. A. C. Malt-Diastase Method

Effect of Pretreatment of Samples

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IN THE production of starch from sweet potatoes at the Laurel Starch Plant, Laurel, Miss., it was found impossible to obtain a starch balance based on the starch content of the raw materials entering the factory and on that contained in the final products—commercial starch, pulp, and waste waters—as determined by the official A. O. A. C. malt-diastase method (*1*). The unknown loss was much greater than could be ascribed to faulty sampling or to any losses which were not being estimated.

In order to demonstrate whether this loss was actual or fictitious, starch was mechanically extracted from potatoes of known weight and determined starch content, with the aid of water and other solutions used at the Laurel plant: sulfurous acid (approximately 0.015 *N*), alkaline sulfite (approximately 0.04 *N* sodium hydroxide and 0.02 *N* sulfur dioxide), and lime water (approximately 0.02 *N* calcium hydroxide). As lime water had been used exclusively during the previous two operating seasons at the plant, particular attention was given in this study to this solution and its effect on the analysis of sweet potatoes and the products derived therefrom.

The mechanical extraction of starch from sweet potatoes in the factory (*5*) is accomplished by grinding the potatoes very finely, mixing the ground mass with water or solution, and separating the starch suspended in the liquid by screening. The washing and screening operations are repeated several times on a countercurrent principle. In these laboratory tests 500 grams of potatoes, ground with a sugar-beet sampling rasp, were thoroughly agitated with 2500 cc. of water or one of the solutions mentioned above, and the starch milk was separated from the pulp with a 200-mesh screen. This operation was repeated twice, employing a fresh portion of liquid in each cycle. The starch water obtained from the three extractions was combined and allowed to settle in a rather shallow layer for about 4 hours, after which the supernatant liquid was siphoned off and allowed to settle further overnight. The first crop of starch was collected in a Büchner funnel and washed, then transferred to a drying tray, where it was dried at about 45° C. The dried starch was weighed and analyzed. The second settlings were simply collected and the entire amount was subjected to analysis. The residual pulp was dewatered by filtering in a Büchner funnel, after which it was weighed and analyzed.

These products were analyzed by the A. O. A. C. malt-diastase (*1*) method, with very slight modifications. As it was thought that the potatoes and pulp were sufficiently disintegrated for the determination of starch, the samples were simply weighed out, transferred to Gooch crucibles, and washed with water until sugar-free. Ordinarily from 100 to 150 cc. of water were used in this step. The contents of the crucibles were transferred to beakers, the starch was gelatinized by boiling for 15 minutes on a hot plate, and 25 cc. of malt diastase, prepared by digesting 10 grams of freshly ground barley malt grain with 150 cc. of water for at least 1.5 hours were added and allowed to act for 1 hour at 55° C. The boiling and malt treatment were repeated as specified. After the malt conversion, the solutions were acidified with 5 to 8 drops of glacial acetic acid to aid clarification, cooled, made up to 250 cc., and filtered; 200 cc. of the filtrate were digested with hydrochloric acid for 2.5 hours on a steam bath, cooled, neutralized, and made to a final volume of 500 cc. Reducing sugars were determined in the resulting solutions by the Lane-Eynon titration method (*3*), employing 10 cc. of Fehling's solution. For determination of the blank, a double portion of the malt extract—100 cc.—was digested with hydrochloric acid and made up to a final volume of 500 cc. It was later found that this procedure for determining the blank, although specified by the A. O. A. C. method, introduced a slight error, which, however, did not alter the conclusions based upon these data. The error in the blank arises from the fact that heating the malt extract, acidifying, and filtering apparently remove from solution something which possesses a slight reducing action after acid digestion.

Applying these extraction and analytical procedures to several lots of sweet potatoes, an average unaccountable loss of 4.2 per cent of the starch in the potato was obtained with water extraction, 7.3 per cent with lime water, 7.1 per cent with alkaline sulfite, and 3.1 per cent with sulfurous acid. With the exercise of every possible precaution to prevent appreciable loss of starch, the only logical conclusion was that the methods of analysis were faulty; otherwise a rather constant unknown loss would be expected, regardless of the solution used in aiding the extraction of starch from potatoes. It would not seem logical to obtain a progressively larger unknown loss of starch in changing the reaction of the liquid phase from acid to alkaline. It was certain that the alkaline solutions did not dissolve any starch and there was no evidence that the actual loss of starch in the alkaline waste waters was any greater than in the acid waters.

In view of such consistent results, it was decided to pretreat duplicate samples of potatoes taken for analysis, in the last two extraction experiments, with the reagents used for the extractions—water, 0.02 *N* calcium hydroxide, and alkaline sulfite (0.04 *N* sodium hydroxide–0.02 *N* sulfur dioxide). This treatment was made after samples were weighed out and prior to washing them in the Gooch crucible. The starch values obtained were rather surprising. The samples given

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only a water treatment indicated 19.54 per cent starch, while those treated with lime gave 18.12 per cent and with the alkaline sulfite, 18.33 per cent.

When these data were used in making the starch credit for the individual extractions, the unaccountable losses became negligible in case of lime water and alkaline sulfite but with water amounted to 3.8 per cent in comparison with an average of 4.2 per cent for the complete series of eight experiments employing water.

Table I. Average Starch Value of Pretreated Sweet Potato Samples

Treatment	Starch Value	Deviation from Starch Value after Water Treatment
	%	%
Water	22.99	...
$Ca(OH)_2$, 0.02 N	21.98	−4.4
$Ca(OH)_2$, 0.04 N	21.83	−5.0
NaOH, 0.02 N	22.05	−4.1
NaOH, 0.04 N	21.98	−4.4
Alkaline sulfite { 0.04 N NaOH, 0.02 N SO_2	22.01	−4.3
Alkaline sulfite { 0.08 N NaOH, 0.04 N SO_2	22.13	−3.8
Sulfurous acid, 0.015 N	23.02	+0.1
Sulfurous acid, 0.03 N	22.99	0
Alcohol (80%)-ether	23.06	+0.3

These results suggested a series of experiments in which duplicate 5-gram samples of rasped potatoes were treated with 100 cc. of two concentrations each of calcium hydroxide, sodium hydroxide, alkaline sulfite, and sulfur dioxide for 1 hour and were compared with duplicates of the same samples treated with water and with those preserved with 80 per cent alcohol and set aside for some time before analysis. All the samples, except the last, were washed with 100 cc. of water after being transferred to Gooch crucibles and finally with a portion of alcohol. The samples preserved with alcohol were transferred to a Gooch crucible and washed with 80 per cent alcohol and finally with ether. The procedure of gelatinization and conversion with malt and acid was the same as described.

The average starch values obtained with six lots of sweet potatoes (four different varieties) analyzed in this manner are presented in Table I.

For all practical purposes, sulfur dioxide and alcohol gave results identical with water, while the alkaline solutions may be grouped together, with calcium hydroxide having slightly the greatest effect. In case of calcium hydroxide, the action responsible for the differences in starch value from that of the water-treated samples appears to be a function of its concentration, increasing as the concentration of the alkali is increased. The maximum effect with 5-gram samples of potatoes was obtained when they were treated with 100 cc. of about 0.04 N calcium hydroxide solution. In later tests where barium hydroxide was substituted for calcium hydroxide, similar results were obtained, but increasing the concentration of the alkali above 0.04 N had no additional effect. Increasing the time of the action of 0.04 N calcium hydroxide from a few minutes to 2 hours, either at room temperature or at 55° C., did not alter the results beyond experimental errors.

These findings are of primary importance to all analysts making starch determinations and raise the questions of how much dependence can be placed on any methods for determining starch in plant materials, what causes these differences, and just what criteria should be selected to determine the accuracy of these methods. It is obvious that many values can be obtained on a given sample with a single analytical procedure, depending upon treatments to which the sample was subjected prior to analysis.

Reasons for Deviations

In attempting to explain the reasons underlying this effect of calcium and other compounds on the determination of starch in sweet potatoes, a number of possibilities come to mind.

1. Compounds of calcium and other alkaline earths affect the reducing-sugar determination with Fehling's solution, but since sodium hydroxide exhibited an effect similar to calcium hydroxide, the amount of the latter which remained unwashed from the plant tissue could not have an appreciable effect upon the determination of dextrose itself. In fact, as much as 5 cc. of 0.04 N calcium hydroxide added to 100 cc. of malt extract did not change its starch value. It had long been observed that pretreatment of sweet potatoes, or pulp, with an alkali had a clarifying action on the malt-digested solutions. Such solutions filtered easily and the filtrates became sparkling clear, while water- or acid-treated samples always remained more or less opalescent and slow to filter. It has not yet been determined, however, what significance to ascribe to this behavior and appearance of the samples at this stage of the analytical procedure.

2. There might be some constituent soluble in alkaline solutions and thus removed from the potato sample which, if not eliminated, would be converted into reducing sugars and thus estimated as starch. Examination of extracts prepared by treating sugar-free samples of potatoes with weak solutions of calcium or sodium hydroxide, however, failed to reveal the presence of any substance having appreciable reducing action either before or after conversion with hydrochloric acid.

3. Since enzymes are sometimes easily affected by certain chemicals, pretreatment of the samples with calcium or sodium hydroxide might prevent the normal action of malt diastase on starch. However, starches (commercial) alone are unaffected by a pretreatment and no evidence has been found that malt diastase is unable to convert starch of the sweet potato if it is available under the existing conditions. Tissues which have been treated with calcium hydroxide do appear, however, to disintegrate with greater difficulty on boiling, so that a few unconverted starch granules are always found in the residue, when examined microscopically, even after a

Table II. Effect of Pretreatment[a] with Chemicals on Starch Values of Sweet Potatoes

Salts (0.04 N)	Starch Value	Deviation from Value with Water	Alkalies (0.04 N)	Starch Value	Deviation from Value with Water
	%	%		%	%
(Water)	23.02	...	(Water)	22.73	...
NaCl	22.65	−1.6	NH_4OH	22.34	−1.7
$BaCl_2$	22.33	−3.0	NaOH	22.06	−2.9
$NaCl_2$	22.41	−2.5	KOH	21.90	−3.6
Na_2SO_4	22.57	−2.0	$Ba(OH)_2$	21.67	−4.7
$CaSO_4$	22.41	−2.5	$Ca(OH)_2$	21.83	−4.0
$Al_2(SO_4)_3$	22.49	−2.3			
$NaHCO_3$	22.49	−2.3	Acids		
Na_2CO_3	22.49	−2.3	(0.02 N)		
(Water)	22.60	...	(Water)	22.60	...
KH_2PO_4	22.60	0	HCl	22.60	0
NaOCl			H_2SO_3	22.60	0
(0.025 gram available Cl_2 per 100 cc.)	22.11	−2.1	H_3PO_4	22.52	−0.5
			Acetic	22.60	0
			Oxalic	22.68	+0.5
			Citric	22.45	0.6

[a] Pretreatment consisted of adding 100 cc. of solution to 5-gram samples and allowing to stand 1 hour at room temperature before transferring to Gooch crucibles and washing with water.

Table III. Starch Values of Sweet Potatoes and of Pulp after Removal of Starch with Water

Expt. No.	Product	Starch Value after Treatment: H_2O	Starch Value after Treatment: 0.04 N $Ca(OH)_2$	Difference	Deviation from Value with Water
		%	%	%	%
1	Sweet potato	24.19	23.16	1.03	−4.3
	Sweet potato pulp	11.90	8.94	2.96	−25.8
2	Sweet potato	25.99	24.65	1.34	−5.2
	Sweet potato pulp	9.90	6.71	3.19	−32.2
3	Sweet potato	22.14	21.16	0.98	−4.4
	Sweet potato pulp (1st extraction)	14.51	11.43	3.08	−21.2
	Sweet potato pulp (2nd extraction)	6.77	4.27	2.50	−36.9
	Sweet potato pulp (3rd extraction)	5.10	2.77	2.33	−45.7
4	Sweet potato pulp[a] (agitated about 45 min.)	4.26	2.42	1.84	−43.1 (moisture 86.01%)
5	Sweet potato pulp[a]	4.02	2.61	1.41	−35.1 (moisture 80.64%)

[a] Coarse particles removed by 20-mesh screen.

double malt treatment. Since sodium hydroxide has an effect similar to calcium hydroxide in the determination of starch but an opposite effect on the physical properties of the pulp, there must be some other explanation for their action. There is not sufficient unconverted starch in the samples pretreated with an alkaline solution to account for the large reduction in their starch value; if present, it could be detected by simple staining with iodine or by microscopic examination, if necessary, for with potatoes the average difference corresponds to 35 mg. of starch per 5-gram sample, while with pulp it corresponds to about twice this amount.

To furnish further proof that unconverted starch was not a factor, samples of sweet potatoes were analyzed with and without further disintegration by grinding with sand. No significant increase in starch values was obtained, indicating that the samples were ground finely enough by the rasp to yield consistent results with potatoes, regardless of pretreatment with calcium hydroxide. In case of pulps resulting from the extraction of starch with water, grinding with sand did seem to increase the starch value of those samples pretreated with lime but not with water. However, this increase probably does not represent liberated starch.

4. The more plausible explanation of the effect of pretreatment upon the starch value of sweet potatoes would seem to be the presence of constituents capable of being converted into reducing compounds, probably sugars, when in their natural condition, by heating or malt diastase and acid digestion; after treatment by free alkalies or by salts, this characteristic is altered to a greater or less extent, depending upon the degree of combination which occurs and the inertness of the resulting compounds. The data in Table II, presented in support of this conception, show that acids apparently have no effect; that alkalies have the greatest effect; and that salts are intermediate in action. The effects of concentration of the various solutions were not established except with calcium and barium hydroxides. Concentrations of the reagents were kept low, to be certain that raw starch itself was not affected by them.

Because those constituents of the sweet potato, other than starch, which contribute to its starch value, appear to be fairly insoluble in cold water or the reagents used in this study, one would expect to find much greater effects caused by pretreatment in the analysis of the residual pulp after extraction of starch with the aid of water. Examination of the data given in Table III reveals this to be the case.

The extraction experiments indicate a loss of substances which interfere with starch determination in the waste waters; otherwise an appreciable unknown loss would not have been obtained when either water alone or a solution of sulfurous acid was used to remove starch from the ground potato. Treating samples for an hour or longer with water at 55° C. caused a reduction in the starch value of both potatoes and pulp, aside from any likely conversion and loss of starch caused by the naturally occurring diastase.

Pretreatment of samples with alkaline solutions prevents to a very slight degree complete conversion of starch with malt, apparently because starch from such treated material is not so readily freed by boiling. Although grinding potato samples with sand after pretreatment did not increase starch values beyond experimental errors, the effect of additional grinding of pulp samples was variable, depending upon whether the grinding was done before or after treatment with lime. The data indicate that the combination of calcium hydroxide with these compounds is not always completed by the simple treatment usually given the samples, and that increase in the starch value is largely due to the liberation of some of the material in an uncombined condition in which it is available for extraction or conversion by malt diastase, rather than to liberating additional starch. The reaction between calcium and these constituents seems to be easily affected—for instance, neutralization of the excess alkalinity with acetic acid just prior to transferring and washing the samples in the Gooch crucible appreciably raised the starch value and neutralization was less effective if the samples were treated with calcium hydroxide after being more finely ground

Table IV. Starch Values of Sweet Potatoes (Obtained by Different Diastase Preparations with A. O. A. C. Procedure)

Expt. No.	Enzyme Preparation	Enzyme Used per sample (Grams)	Starch Value after Treatment with: Water	Starch Value after Treatment with: 0.04 N $Ca(OH)_2$	Deviation from Value with Water	Iodine Reaction of Solution after Enzyme Conversion
			%	%	%	
1	Malt	≈3.3 grams grain	22.16	20.92	−5.6	
	Amylase	0.1	22.14	21.43	−3.2	
	Taka-diastase	0.1	21.77	21.34	−2.4	
2	Malt	≈3.3 grams grain	23.75	22.81	−3.9	
	Amylase	0.05	23.54	22.72	−3.5	
3	Malt	≈3.3 grams grain	23.52	22.33	−5.1	Water and $Ca(OH)_2$ samples, both yellow
	Amylase	0.005	...	21.03	...	$Ca(OH)_2$ sample, blue
	Amylase	0.010	...	20.90	...	$Ca(OH)_2$ sample, blue
	Amylase	0.020	...	21.76	...	$Ca(OH)_2$ sample, blue
	Amylase	0.050	23.36	22.19	−5.0	Water sample, yellow; $Ca(OH)_2$ sample, reddish purple
	Amylase	0.100	...	22.19	...	$Ca(OH)_2$ sample, red

with sand; the use of a large dosage of malt extract slightly increased the starch values of pretreated pulp.

In order to obtain further proof that some constituents of sweet potatoes other than starch contribute to the starch value determined by malt diastase, amylase (Wallerstein's) and taka-diastase were substituted for it, but otherwise the same procedure was used. Comparisons were made on the assumption that these preparations are mixtures of enzymes in varying proportions and that different results would be obtained after the same pretreatment if the variation in apparent starch value is due to the converting action of the enzymes. On commercial starch these enzymes gave identical values, within analytical errors, but in the analysis of sweet potatoes gave somewhat variable results (Table IV).

The low starch values obtained with less than 0.05 gram of amylase were apparently due to incomplete conversion of starch into a fully soluble form. In all these samples calcium hydroxide reduced the degree of conversion by amylase, judging from the iodine reaction. This is doubtless caused, to a certain extent, by an increase in pH above the optimum; these solutions were not buffered but the mere presence of calcium seems to possess a slight retarding action. In the case of malt diastase there was either sufficient buffering action or sufficient enzyme concentration to overcome any appreciable retarding action of the calcium compound, for the reaction of the converted starch solution was always yellow toward iodine. The pH of the lime-treated samples after conversion with malt diastase was somewhat higher than that of the untreated samples—for example, in case of potatoes, the pH was approximately 7.5 instead of 7.0. As salts themselves affect the starch determination, it is not possible to employ buffer solutions on potatoes and differentiate precisely between their effect and that of hydrogen ions, although it would be extremely desirable to do so.

Limited tests by the official A. O. A. C. method (*2*) have indicated the presence of pentosans, aside from those derived from the malt extract, in the solution obtained from fleshy plant materials after boiling with water and treatment with malt extract at 55° C. It is obvious that the pentosans were derived from the plant material, but no attempt was made to determine whether these became soluble as a result of enzymatic action of the malt extract or as a result of degradation caused by boiling the sample prior to malting. Probably the latter is partially responsible, judging by the relatively high solubility of pectinous material in hot and relatively weak salt solutions—for example, sodium or ammonium oxalate and sodium phosphate–citric acid buffer solutions whose reaction is around neutrality—which, of course, would give pentosan reactions. If such pentosans are present, they will be determined as dextrose and calculated as starch. There are probably other constituents which give pentosan reactions which also contribute to the errors of starch analysis of fleshy plant materials.

The solubility of these constituents is doubtless altered by the treatment accorded the sample prior to digestion. A relatively large proportion appears to be acidic in nature, as they seem to react with bases or salts to form compounds having a lower solubility in water or inactivity toward the enzymes of the malt extract (or both). Treatment of such fleshy plant materials as sugar-beet pulp, carrots, and rutabagas, which contain no starch or at most very little, with lime water prior to analysis reduces their apparent starch content from 58 to 100 per cent below the original value determined by the official malt-diastase method. In cases where pentosan determinations were made, pretreatment of the sample with lime water reduced the apparent pentosan content of the solution after malt treatment from 65 to 75 per cent below the original value. These results (Table V) indicate that pretreatment of vegetable matter with alkalies, particularly calcium (or barium or strontium), materially reduces the error resulting from solubilizing certain constituents which exhibit reducing power after malt and acid conversion and hence are calculated as starch.

Errors of a similar order were also evident in the recently developed polariscopic method (*4*) in which dispersion of the starch is accomplished by small amounts of sodium hypochlorite at boiling temperatures. Pretreatment of the samples with lime water seemed to correct the polarization of the starch solution prepared with the hypochlorite more effectively than it did the reducing power after malt and acid digestion. Further work should be conducted, however, to determine the accuracy of this belief, possibly on starch-free fleshy vegetable matter such as carrots to which pure starch can be added in varying proportions to simulate starchy vegetables.

Table V. Effect of Pretreatment of Fleshy Plant Materials with Calcium Hydroxide

Plant Material	No. of Samples Analyzed	Starch Value after Pretreatment: Water	Starch Value after Pretreatment: 0.04 *N* $Ca(OH)_2$	Difference	Deviation from Value with Water
		%	%	%	%
Sweet potatoes	51	22.25	21.55	0.70	−3.1
Sweet potato pulp	33	7.34	5.89	1.45	−19.8
White potatoes	5	13.04	12.54	0.50	−3.8
White potato pulp	2	7.21	3.97	3.14	−44.9
Dasheen	1	26.91	26.20	0.71	−2.6
Rutabagas	1	0.45	0.15	0.30	−66.7
Carrots	1	0.68	0.00	0.68	−100.0
	1	1.04	0.44	0.60	−57.7
Sugar-beet pulp	1	8.74	0.64	8.10	−92.7
	1[a]	3.19	1.35	1.84	−57.7

	Pretreatment	Pentosan Content of Solution after Malt Digestion
		%
Beet pulp[a]	H_2O	2.75
	$Ca(OH)_2$	0.44
Sweet potato pulp	H_2O	0.43
	$Ca(OH)_2$	0.11

[a] Same sample.

Summary

The data on sweet potatoes indicate that a considerable error in starch value arises with the malt-diastase method when the usual procedure is followed. Pretreatment with calcium or barium hydroxide solution prevents, to a very great extent, the action of malt diastase on certain nonstarchy constituents usually determined as starch, and for the most accurate determination of starch in sweet potatoes such pretreatment is very essential.

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Effect of Soft Glass on Melting Point of Rotenone

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JONES and Wood (*1*) have recently shown that the melting point of α-toxicarol is markedly lower in soft-glass than in hard-glass capillary tubes; one sample that melted at 230–231° C. in Pyrex melted at 205–206° C. in ordinary soft glass. The effect was shown to be due to the greater alkalinity of the soft glass. Rotenone has also been found to exhibit a less marked but definite depression of melting point in soft glass. Pure rotenone melted about 3° lower in ordinary soft glass than in Pyrex and about 5° lower in a more alkaline soft glass (Corning electrode glass No. 015) than in ordinary soft glass.

Table I. Melting Points and Degrees of Purity of Rotenone in Capillary Glass Tubes

Rotenone Sample	In Pyrex Glass: Melting point	In Pyrex Glass: Degree of purity	In Ordinary Soft Glass: Melting point	In Ordinary Soft Glass: Degree of purity	In Corning Electrode Glass: Melting point	In Corning Electrode Glass: Degree of purity
	° C. (cor.)	%	° C. (cor.)	%	° C. (cor.)	%
Pure	163–164	100	159.5–160.5	99.4	155–156	98.6
Impure, sample A	161–162	99.8	157.5–159	99.0	154.5–155.5	98.4
Impure, sample B	151.5–153.5	97.8	149–151	97.2	146.5–148.5	96.6

This effect is important in the analysis of derris and cube roots for their rotenone content. Many workers use the melting point as a qualitative indication of the purity of the rotenone obtained. Furthermore, in the quantitative method of Meijer and Koolhaas (*3*) the melting point of the separated rotenone is used as one means of calculating the purity, and from this the content, of the sample.

The melting points of pure rotenone and of two samples of impure rotenone in Pyrex and in two soft glasses are given in Table I. The glass was cleaned, and melting points were determined by the methods used in the work on α-toxicarol (*1*). From the qualitative standpoint a melting point of 155–156°, the value obtained for pure rotenone in Corning electrode glass, would not be considered to indicate a high degree of purity. Furthermore, the melting points in soft glass are not only lower but less sharp than in Pyrex and thus give every indication of material of lower purity.

By use of a graph given by Koolhaas (*2*), the degree of purity indicated by these melting points was determined, on the assumption that the graph held for the samples used here, and these values are also recorded in Table I. Meijer and Koolhaas (*3*) state that for calculation of purity by this method the melting point should not be less than 140°; hence, samples with lower values have not been included in this work. The maximum difference in apparent purity of any one sample as calculated from the melting points in Pyrex and Corning electrode glass is shown to be 1.4 per cent. This would introduce an error of only about 0.1 per cent in the rotenone content of a 5 per cent rotenone sample and about 0.2 per cent in that of a 10 per cent sample. Although the discrepancy in melting point between Pyrex and soft glass does not introduce a very serious error into results by this method of calculation, it is a recognized source of error which is easily eliminated and should be avoided.

To specify that all melting points should be taken in the same type of soft glass is not sufficient, since the lowering effect, as stated for α-toxicarol, must depend on the degree of contact with the glass, and hence on the size of the crystals and other factors. In all analytical and investigative work dealing with rotenone and related compounds, melting points determined by capillary tube should be made in a glass of low alkalinity such as Pyrex.

It is not intended to imply that the melting-point method of determining rotenone purity is used or recommended by this writer. He merely wishes to point out to those who employ it that use of soft glass for melting point constitutes a possible source of error. As a matter of fact it is his opinion that other sources of error are also present in this method and that purity of rotenone may be more accurately determined by other means.

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Stability of the Permanganate-Periodate Color System

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IN MAKING a spectrophotometric study of the Willard and Greathouse periodate method (*2*) for the colorimetric determination of manganese the writer (*1*) found no evidence whatever of fading or other change in the color of the system over a period of two months.

The solutions which had been used in this test, containing 1.25, 2.50, 3.75, and 5.0 mg. of manganese with 10 ml. of concentrated sulfuric acid and 0.3 gram of potassium periodate per 250 ml., were allowed to stand in glass-stoppered Pyrex bottles in diffuse light for an additional 23 months. Spectral transmission curves were then made and compared with the curves given by the corresponding freshly prepared solutions. From the transmittancy at 522 mμ the percentage error in the apparent concentration of manganese was calculated (*1*) by use of the special color slide rule.

Table I. Stability of Color

Concentration of Manganese	Transmittancy at 522 mμ: Fresh solution	Transmittancy at 522 mμ: Old solution	Apparent Change in Concentration of Manganese
Mg./l.	%	%	%
5	39.5	39.8	−0.7
10	15.2	16.6	−4.6
15	5.9	6.0	−0.5
20	2.0	2.7	−7.5

The results, given in Table I, prove that the color of the permanganate-periodate system is exceptionally stable.

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The Signer Method for Determining Molecular Weights

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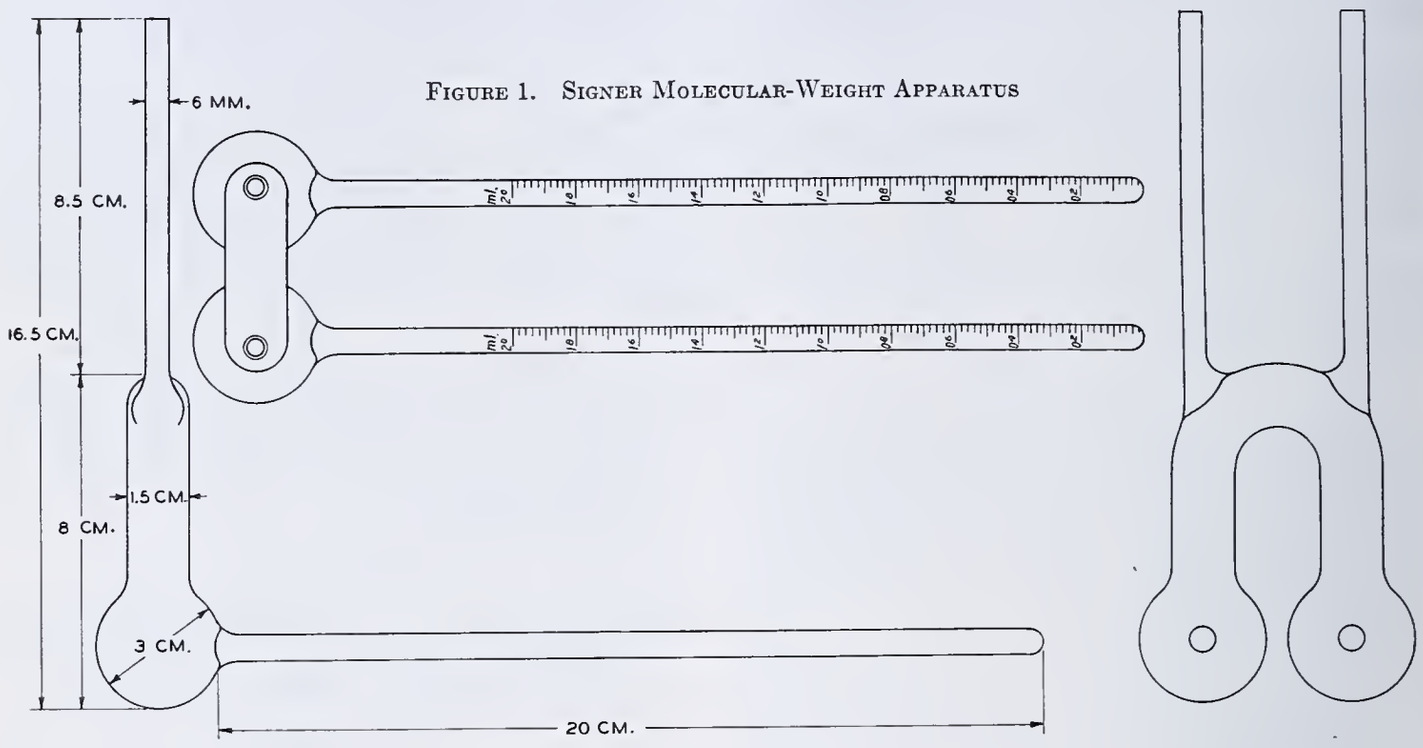

FIGURE 1. SIGNER MOLECULAR-WEIGHT APPARATUS

THE purpose of this communication is to call attention to and elaborate upon an extremely accurate but little known method for determining molecular weights. The procedure published by Signer (*1*) involves the principle of isothermal distillation and has been shown experimentally to be practical. However, the original directions are such that more specific information is desirable. For this reason a workable outline for making the determination is presented.

The experiment consists in permitting two solutions in an evacuated system, with solvent vapors in contact, to arrive at vapor-pressure equilibrium by isothermal distillation. Arrangements must be available for determining the volumes of each solution. The apparatus used to realize this is shown in Figure 1. It is smaller than the original, to make possible accurate measurements of 1.5 to 1.7 cc. of liquid. The solutions usually employed are approximately 0.1 molar, from which it follows that the quantity of substance necessary for a determination is small.

The samples of standard and unknown material, in the form of pellets, are weighed and dropped through the open side arms of the apparatus, so that one bulb receives the standard and the other the unknown. The filling tubes are then constricted near their bases to facilitate subsequent sealing. As soon as the glass cools, 2 cc. of solvent are added to each bulb, after which one tube is sealed at its constriction. As this seal is made, a very gentle stream of dry air should be blown through the tube to prevent vapors of the solvent from coming in contact with the hot glass. The system is then evacuated from a line in which is interposed 1 meter of 1-mm. capillary tubing, and in this manner approximately 0.3 cc. of solvent is distilled from each bulb. While the distillation continues, the constricted part of the connecting tube is sealed with a soft gas-oxygen flame. The closed evacuated system then contains two solutions containing definite quantities of standard and unknown material arranged as outlined above. Therefore, if the entire apparatus is isothermally insulated, solvent will distill from the solution of greater vapor pressure to the one of less, until equilibrium is established. When this occurs the volumes of the two solutions will be constant and equimolar. These volumes may then be read by tilting the apparatus and draining the solutions into the graduated side arms. Five minutes are arbitrarily taken for this purpose.

With the data thus available, it follows from Raoult's law that

$$M_1 = \frac{G_1 M V}{G V_1}$$

where M, V, and G are, respectively, the molecular weight, volume of solution, and weight of the standard, and M_1, V_1, and G_1 are the corresponding values of the unknown. In practice, the volumes are read every 1 to 3 days, depending upon the solvent used, until they become constant. The results thus obtained may be plotted (volume against time) and, if the experiment is progressing normally, smooth typical curves are obtained as presented in Figures 2 and 3.

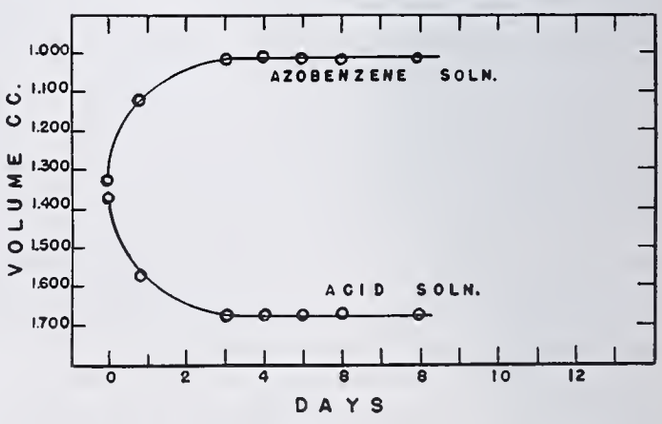

FIGURE 2. AZOBENZENE–*o*-CHLOROBENZOIC ACID

Solvent, ether; solutions 0.1416 molar at equilibrium. Molecular weight of *o*-chlorobenzoic acid, 156.5; found, 158

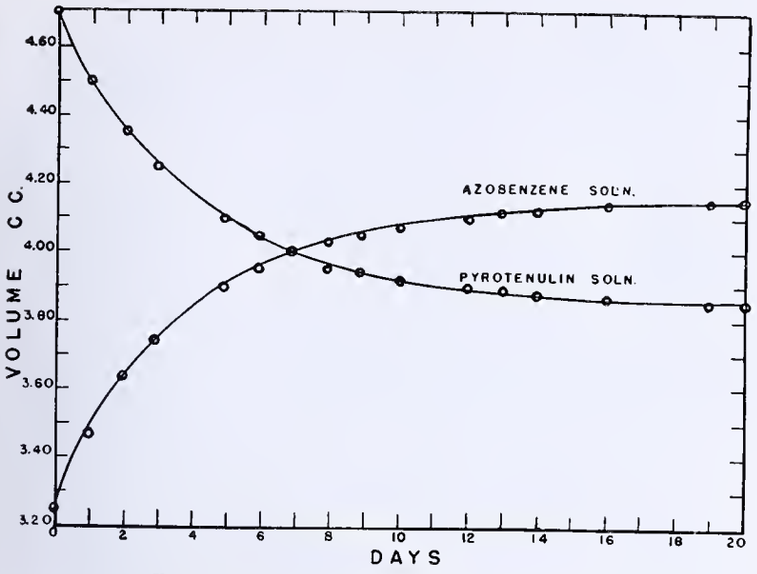

FIGURE 3. AZOBENZENE-PYROTENULIN
Solvent, chloroform; solutions 0.1367 molar at equilibrium. Molecular weight of pyrotenulin, 288.3; found, 288.4

The essential experimental factor in this determination is the maintenance of the apparatus in an isothermal condition. A very simple way to do this is to conduct the experiment at room temperature in a heavy metal container, such as an aluminum pressure cooker, which has a high thermal conductivity. Under these conditions the time necessary for a pair of solutions to reach equilibrium is greater than at elevated temperatures, but the simplicity of the procedure and the accuracy of the results warrant its use. The duration of the experiment is also dependent upon the concentration of the solutions, their relative molarity when prepared, and the solvent used. The best solvents are those with high vapor pressures. Ether, acetone, ethyl bromide, and chloroform have been employed, but others would undoubtedly be as good. These factors can be judged by a study of the curves in Figures 2 and 3, where ether and chloroform are the respective solvents. Azobenzene is an excellent standard where organic solvents are used. It is easily purified, is permanent in the air, and is readily soluble in most solvents, and the color of its solution distinguishes it from the unknown.

TABLE I. MOLECULAR WEIGHTS DETERMINED BY METHOD DESCRIBED

Substance	Molecular Weight Found	Molecular Weight Calculated
α-Nitronaphthalene, $C_{10}H_7NO_2$	173.7	173.1
Rotenone, $C_{23}H_{22}O_6$	393.3	394.3
2-(3-Chlorobenzoyl)-benzoic acid, $C_{14}H_9O_3Cl$	260.7	255
o-Chlorobenzoic acid, $C_7H_5O_2Cl$	156.5	157.4
Pyrotenulin, $C_{17}H_{20}O_4$	288.3	288.4
Isotenulin, $C_{17}H_{22}O_5$	304	306.4
Ferulic acid, $C_{10}H_{10}O_4$	195.5	198.1
p,p'-Dibromodiphenyl, $C_{12}H_8Br_2$	313	312

The procedure as outlined has given uniformly good results with all classes of compounds studied and appears to be generally applicable. It is thus one of the best methods available for the determination of molecular weights. Several results obtained by it are presented in Table I.

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Determination of Hydroxyl Content of Organic Compounds

Acetyl Chloride as a Reagent

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THE simplest methods for the determination of the hydroxyl radical in organic compounds are those based on esterification procedures. Acetic anhydride with and without pyridine has been widely used in this connection (*2*, *3*, *4*, *6*), but although acetyl chloride is a much more active acetylating agent, little attention has been given to the possibility of using it in quantitative work (*5*). It is difficult to measure and handle small amounts of this reagent.

Smith and Bryant (*5*) were the first to recognize its possibilities and employ it for analytical purposes. These workers avoided the problem of volatility and activity by employing it in the form of an acetyl pyridinium chloride suspension in toluene; in other respects their method was similar in principle to the acetic anhydride–pyridine procedures. In this indirect use of acetyl chloride it is probable that ketene (*8*) was the acetylating agent.

Much would be gained if pure acetyl chloride rather than a suspension of acetyl pyridinium chloride in toluene were used as the esterifying agent. This would eliminate the annoying pyridine vapors, the diluent and solubility effects of the toluene, and the use of a solid acetylating agent.

In order to use pure acetyl chloride a way must be found to measure and control it. Acetyl chloride reacts very slowly at low temperatures even with methanol; consequently solid carbon dioxide offers a convenient means of controlling its reaction rate. Linderstrom-Lang and Holter (*1*) have described an ingenious pipet for the precise measurement of small volumes. Using a modified form of this instrument, a simple technique has been developed for using pure acetyl chloride in determining the hydroxyl content of organic compounds.

Experimental

REAGENTS. Acetyl chloride, Eastman (practical), sodium hydroxide, 0.3 *N* (carbonate-free), phenolphthalein indicator, and dry ice.

FIGURE 1. DIAGRAM OF PIPET

APPARATUS. The pipet illustrated diagrammatically in Figure 1 was constructed from 10-mm. Pyrex tubing, was 21 cm. over-all, and delivered 0.5 ml. of liquid. Its two important features were the 0.1-mm. constriction and the split rubber cap. By pressure parallel to the slit, the cap functioned as a medicine dropper. When a slight pressure was exerted in the opposite direction, the slit opened and the contents were then free to drain.

The pipet was filled in the manner of a medicine dropper, the slit was opened, and the contents were drained to the constriction. The acetyl chloride could now be transferred to the reaction vessel. In delivery, by opening the slit, the contents of the pipet were allowed to drain into the lower capillary and then a slight pressure was exerted on the closed rubber cap, slowly forcing all the liquid out of the pipet.

The reaction vessel (Figure 2) was constructed from a 20-cm. (8-inch) and a 10-cm. (4-inch) test tube. An inverted 15-cm. (6-inch) test tube fitted with a glass hook served to seal the water trap.

ANALYTICAL PROCEDURE. Samples of 0.1 to 0.750 gram (depending on the compound) were weighed in a 10-cm. (4-inch) test tube which in case of volatile materials was stoppered during the weighing. The test tube was then immersed in dry ice. After the charge was sufficiently chilled, a measured charge of acetyl chloride was added with the pipet and the small test tube was placed in a 20-cm. (8-inch) test tube containing 5 to 10 ml. of water. As indicated in Figure 2, a 15-cm. (6-inch) test tube inverted over the smaller test tube served as a seal. The tube was then stoppered and placed in a bath at 40° C. for 20 minutes. In many cases this was unnecessary, since reaction took place immediately on coming to room temperature.

After 20 minutes the reaction vessel was inverted to hydrolyze the excess acetyl chloride. The contents were carefully removed and titrated with standard sodium hydroxide, using phenolphthalein as the indicator. A small amount of alcohol was used to wash the last traces of ester from the reaction flask. The per cent of hydroxyl present was then calculated by means of the simple equation

$$\frac{(\text{Blank} - \text{ml. of sodium hydroxide}) \times \text{normality} \times 17}{10 \times \text{weight of charge}} = \%$$

Because of the quality of acetyl chloride used and because its titer may change with temperature, it is necessary to make blank determinations simultaneously. These blank runs also serve as a good check on the analyst's precision. Whenever fading of the end point occurred, the base was added in small increments (0.03 ml.) until the color persisted for 30 seconds.

Results and Discussion

The results obtained with this procedure are given in Table I.

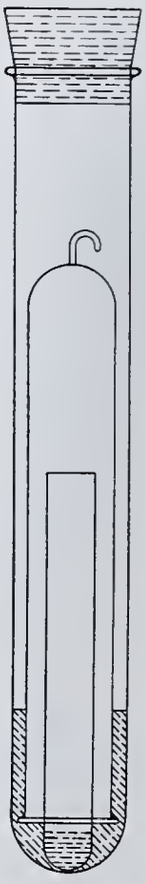

FIGURE 2

TABLE I. ACETYLATION OF ALCOHOLS AND PHENOLS

	No. of Detns.	Per Cent of Theoretical Hydroxyl Content	Average Deviation
Primary and secondary alcohols			
Methanol	4	95.4	0.3
Ethanol	5	95.7	0.2
Propanol-1	4	95.4	0.3
Propanol-2	4	95.4	0.1
Butanol-1	5	98.5	0.5
2-Methylpropanol-1	5	98.9	0.3
Butanol-2	3	98.2	0.1
Pentanol-1	5	96.1	0.3
3-Methylbutanol-1	5	100.1	1.0
Pentanol-3	5	99.0	0.2
Hexanol-1	5	93.9	0.2
Cyclohexanol	5	99.7	0.2
2-Ethylhexanol-1	5	100.0	0.1
Octanol-2	5	98.6	0.2
Lauryl alcohol	4	97.2	0.8
Benzyl alcohol	5	101.0	1.0
Furfuryl alcohol	5	[a]	
Cinnamyl alcohol	5	99.0[a]	1.2
Tertiary alcohols			
2-Methylpropanol-2	5	49.0[b]	1.9
2-Methylbutanol-2	5	88.7[b]	2.4
Benzopinacol	1	3.5[c]	
Polyhydric alcohols			
Glycerol	4	98.8	0.4
Ethylene glycol	4	99.1	0.3
Propylene glycol	4	99.0	0.4
Diethylene glycol	5	99.0	0.4
Mannitol	3	93.4[d]	3.2
Substituted alcohols			
Ethylene chlorohydrin	5	98.6	0.2
1,3-Dichloropropanol-2	4	100.3	0.3
Terpenes			
Borneol	5	100.9	0.1
Menthol	5	100.7	0.3
Geraniol	5	140.4[e]	4.5
Linaloöl	5	131.0[e]	1.5

Interfering factor:
[a] Poor end point, unable to titrate.
[b] Reacts with evolved hydrogen chloride.
[c] Rearrangement.

	No. of Detns.	Per Cent of Theoretical Hydroxyl Content	Average Deviation
Miscellaneous			
Lithium lactate	3	102.2	1.5
Rochelle salt	3	65.8[d]	1.7
Citric acid	3	38.3[d]	3.1
Benzoin	2	124.1[f]	3.2
Phenols			
Phenol	5	99.4	0.2
o-Cresol	5	100.6	1.1
m-Cresol	4	99.9	0.2
p-Cresol	5	101.0	0.5
p-*tert*-amyl phenol	2	101.2	0.1
α-Naphthol	2	107.7[e]	4.5
β-Naphthol	2	110.6[e]	5.5
Thymol	5	99.5	0.7
Xylenol	2	99.3	0.3
Catechol	4	100.1	0.5
Hydroquinone	2	98.2	0.3
Resorcinol	2	98.7	0.3
Orcinol	2	98.0	1.4
Phloroglucinol	5	[a]	
Pyrogallol	2	98.7	0.8
Substituted phenols			
Guaiacol	5	99.7	0.5
Isoeugenol	5	137.2[e]	4.1
Vanillin	5	[a]	
p-Chlorophenol	3	101.0	0.4
o-Chlorophenol	5	99.2	0.5
2,4-Dichlorophenol	2	96.5	0.1
3-Bromo-4-phenylphenol	2	100.6	1.3
2,4,6-Triiodophenol	1	8.1	
o-Nitrophenol	2	100.9	1.1
m-Nitrophenol	2	103.1	2.1
p-Nitrophenol	2	100.0	0.9
2,4-Dinitrophenol	2	9.5	0.5
2,4,6-Trinitrophenol	5	1.6	
3,5-Dinitro-*o*-cresol	2	[a]	
Salicylic acid	2	3.3	
m-Hydroxyl benzoic acid	2	61.9	4.3
p-Hydroxyl benzoic acid	2	89.8	6.5
Methyl salicylate	5	14.8	3.1
α-Nitroso-β-naphthol	2	[a]	

[d] Solubility.
[e] Possibly addition with evolved hydrogen chloride.
[f] Enolization.

This method is rapid and lends itself to mass production methods. It has considerable applicability and gives as good a precision as older methods based on the use of an acetylating agent. In the case of the phenols the results in general were superior. From the results in Table I it is apparent that several limitations are imposed by the character of the molecule undergoing esterification.

The effect of solubility in this connection has been noted by others (*5*). In initial experiments with mannitol low results were obtained. When a small amount of water was added to this compound the acetylation was almost complete. The interference of several functional groups—for example, aldehyde and nitroso—with indicators was observed. This made titration by the usual methods impossible.

Side reactions involving the liberated hydrogen chloride may account for several unusual results, particularly in the case of such olefinic compounds as geraniol, linaloöl, and isoeugenol, and may explain the unusual behavior of alpha- and beta-naphthols in which one ring readily forms addition products (*7*). The data obtained with tertiary alcohols can also be explained on this basis, since such alcohols are known to react readily with hydrogen chloride (*7*).

Interesting observations were noted in the studies of the substituted phenols. As indicated in Table I, the more acidic phenols such as picric acid were not acetylated. This was to be expected, as the hydroxyl group is more acidic than phenolic in character and therefore behaves as an acid (*7*).

The failure to acetylate salicylic acid and its isomers cannot be adequately explained on this basis; perhaps it is more a question of chelation. It is evident that such factors as solubility, chelation, unsaturation, enolization, and rearrangement are important in determining the hydroxyl content of organic compounds by the use of acetyl chloride.

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A Method of Installing Tube-Wall Thermocouples

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THE determination of heat transfer coefficients through liquid films requires a method of measuring tube-wall temperatures.

McAdams (*4*) gives an excellent discussion of the measurement of surface temperature, citing the literature on the subject. Colburn and Hougen (*3*) also discuss the errors resulting when thermocouple junctions are installed directly on the surface or when the leads are brought out through a medium either hotter or colder than the junction. All workers agree that it is advisable to bring the leads from the thermocouple junctions through a substantially isothermal zone (as through the metal wall itself rather than through the fluid stream), but McAdams states that the difficulty of construction has probably prevented wide use of this method.

Rietschel (*6*) installed the thermocouple junctions in grooves in the wall of a pipe or tube and brought the leads out in grooves covered over with litharge and glycerol cement. Reiher (*5*) and Colburn and Hougen (*3*) also used a groove installation and depended on litharge and glycerol cement for insulation, although the leads were finally brought out through the hot vapors.

Baker and Mueller (*2*) used an installation similar to that of Colburn and Hougen, in that a groove was cut three quarters of the distance around the tube and the junction was installed in a drilled hole at the end of the groove. The leads were sealed into the groove and finally carried out through the hot vapors. Various methods of sealing the lead wires into the groove were tried by Baker and Mueller who state that "pure Bakelite varnish plus a filler had a tendency to shrink and crack in service. Litharge and glycerol cement disintegrated. Litharge and glycerol cement with a covering of the Bakelite varnish also failed." This was probably due to the fact that they condensed vapors of organic compounds as well as steam. In the method finally adopted, they enclosed the leads in small brass tubes and sealed them into the grooves with solder which was polished until flush with the surface of the heat transfer tube. However, the leads were brought out of the condenser shell through the hot vapors.

Akin and McAdams (*1*) installed thermocouple junctions in holes drilled tangentially in the wall of the heat transfer tube and insulated the lead wires with Pyrex capillary tubing.

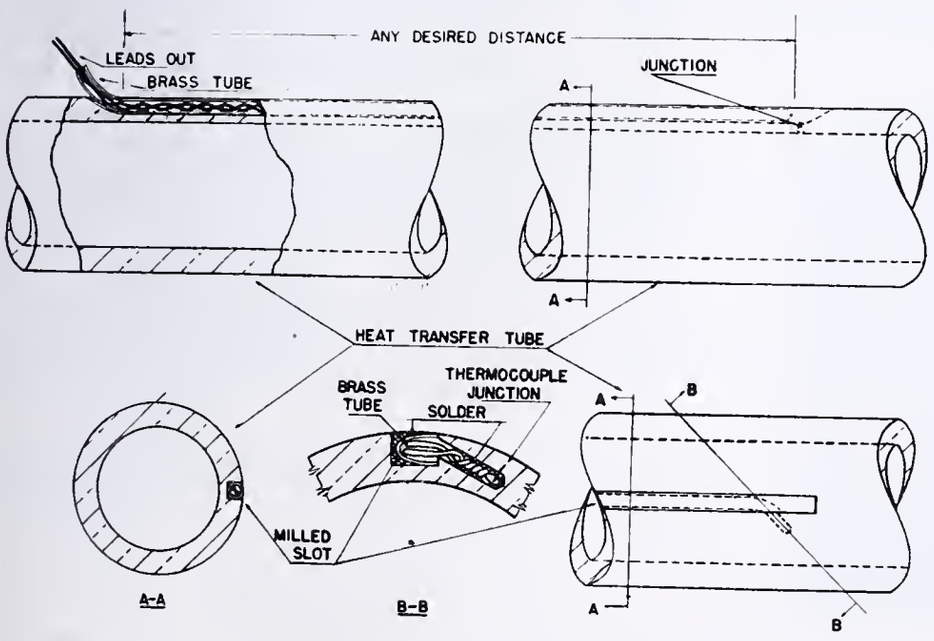

FIGURE 1. DETAILS OF INSTALLATION

In investigating the heat transfer coefficients for the condensation of mixed vapors of turpentine and water on a single horizontal tube, the authors sought a method of tube-wall thermocouple installation which would meet the following stipulations: (1) lead wires to be brought out entirely through a substantially isothermal zone; (2) no insulation to be exposed to turpentine, which readily attacks and softens litharge-glycerol cement and similar materials; (3) con-

struction to be sufficiently simple to be performed in the average machine shop at moderate cost.

The method which was finally worked out apparently satisfies these conditions.

Details of the installation appear in Figure 1, which shows only the tube and the method of installing the thermocouple junctions in the tube wall. This tube could be that of a single-tube test condenser, liquid-to-liquid heat exchanger, evaporator, or other piece of experimental equipment where the measurement of tube-wall temperature is necessary. The tube used in this work was 1-inch, extra-heavy copper pipe.

A slot is milled in the outside wall of the tube, parallel to the longitudinal axis, from the point at which the junction is to be installed to a point where the leads may be brought out without influencing the transfer of heat or the flow of fluids. This operation is easily performed in a well-equipped machine shop. The junction is installed in a 0.062-inch hole drilled 0.25-inch into the tube wall at an angle from the slot. The junction is inserted in this hole and soldered in place. The leads are enclosed in a small brass tube and laid in the slot. The brass tube is bent slightly at the end and butted against the wall of the slot, in such a way that it completely covers the leads. The slot is then filled in with solder and is polished down to the original contour of the tube. The size of the slot depends on the size of the brass tubing necessary to enclose the leads. For a pair of No. 24 enameled, silk-covered wires, a brass tube 0.093 inch in outside diameter and 0.071 inch in inside diameter will be found satisfactory. This tube will fit nicely in a milled slot about 0.10 inch wide and 0.10 inch deep. By making the slot wider, two or three sets of leads may be brought out from each end of the tube, and if more than four to six couple installations are desired in one tube wall, the number of slots may be increased.

This method of installation may be used to locate a thermocouple junction at any point in the wall of the tube and the leads may be carried to any desired point through a substantially isothermal zone. The possibility exists that the solder, having surface characteristics different from those of the tube metal, might influence the flow of condensate over the surface of the tube. This effect could be eliminated by plating the surface of the tube after the thermocouple installation. If the heat flux is high, a correction should be made for the temperature drop between the tube surface and the point of junction installation. This can easily be done when the heat flux and the thermal conductivity of the tube metal are known.

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Small-Scale Centrifuge Accessories for Use with Corrosive Materials

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THE purification of 0.45 to 2.27 kg. (1 to 5 pounds) of reagent chemicals by recrystallization from strong acid or alkaline solutions presents a special problem in apparatus assembly. Where the liquids involved are not particularly corrosive Johnson and Miller (*1*) suggest the use of centrifuge cups and receivers machined from Lucite. The present description involves an improvement, since standard parts are involved and possible interaction of equipment and reagents is eliminated.

The apparatus is shown in Figure 1, mounted on an International centrifuge size 2 (International Equipment Company, 352 Western Ave., Boston, Mass.). In the center foreground is a half section which illustrates the assembly of trunnion cup No. 373 with rubber cushion insert No. 582 and bottle No. 558, cut into two parts and the top discarded. The special centrifuge cups were made by the Coors Porcelain Co.

Figure 1. Apparatus

The centrifuge cups are 8.57 cm. (3.375 inches) tall with an outside diameter at the base of 4.13 cm. (1.625 inches) and at the top of 5.72 cm. (2.25 inches). The inside diameter at the top is 5.4 cm. (2.125 inches) and the upper flange is 1.75 cm. ($^{11}/_{16}$ inch) wide and is offset 0.32 cm. (0.125 inch) to provide a shoulder support on top of the trunnion cup. The inside depth is 8.26 cm. (3.25 inches) and the capacity is 170 ml. The cups are glazed inside and outside except for the bottoms. There are 190 to 200 perforations in the bottom, all less than 0.05 cm. (0.02 inch) in diameter. A second offset 3.33 cm. ($1^{5}/_{16}$ inches) from the top is 0.16 cm. ($^{1}/_{16}$ inch) in depth. The lids are standard size Coors No. 4 crucible lid. The cup and lid weigh 177 grams (6.25 ounces).

The filter flask assembly in the background is for use in removing excess mother liquor by reduced pressure filtration. The filter flask is of 4000-ml. capacity provided with a 10.16-cm. (4-inch) 60° funnel. The support for the centrifuge cup is made according to the directions of Smith and Gring (*2*) from a No. 14 rubber stopper in which a 5.08-cm. (2-inch) hole is cut and the stopper then turned inside out.

The assembly as described will provide for 0.45 kg. (1 pound) of crystals of average density and the equipment can be whirled at 2000 r. p. m. supported in trunnion No. 236 without danger of perforating the flat bottom of the centrifuge cup. By changing the receiving cups and using a selected liquid, the material being purified may be conveniently washed.

The cost of the only special part, the perforated porcelain cup, was $2.50. Ten pounds of material can be easily filtered, centrifuged, and washed in one hour by the use of this equipment.

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Europium and Ytterbium in Rare Earth Mixtures

Polarographic Determination

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IT IS well known that europium and ytterbium differ from the rest of the rare earth metals in their relative ease of reduction from the trivalent to the divalent state. Bruckl and Noddack (*1, 12*) have determined the reduction potentials of various rare earth sulfates in 0.01 *M* solution, in the absence of indifferent electrolyte, using the dropping mercury electrode. In each case, two polarographic waves were obtained, indicating a two-step reduction, first to the divalent ion and then to the metal. Their results suggested the possibility of determining either europium or ytterbium, or both simultaneously, in rare earth mixtures.

Holleck (*3*) has proposed an indirect polarographic method for the determination of europium, involving addition of a known concentration of zinc to the rare earth solution and calculation of the europium concentration from the relative wave heights obtained for europium and zinc. Such a method tacitly assumes that the zinc ion and the europic ion have equal diffusion coefficients. Owing to the larger size of the hydrated europic ion and its higher charge (*8*), its diffusion coefficient would be expected to be lower than that of the zinc ion. It is shown below that Holleck's method is only approximate.

No polarographic methods have previously been suggested for the determination of ytterbium.

It was first necessary to determine the current-voltage curves of pure europium and ytterbium in the presence of an excess of suitable indifferent electrolyte to establish a basis for the detection and determination of these elements. Ammonium chloride was chosen as the indifferent electrolyte, because it provides a sufficiently acid medium to prevent the hydrolysis of dilute rare earth chlorides without causing interference with the ytterbium wave due to hydrogen evolution. Moreover, ammonium chloride is very easily removed from the samples, allowing a simple recovery of the rare earth material.

Current-Voltage Curves of Europium

A sample of pure europium oxide, one of a series prepared by fractional crystallization for use in atomic weight determinations, was kindly provided by B. S. Hopkins. A 0.1-gram sample, weighed after ignition to 700° C. for 5 hours, was dissolved in 2 ml. of 6 *N* hydrochloric acid and made up to 100 ml. in a volumetric flask. Various portions were pipetted into 50-ml. volumetric flasks, titrated with 0.5 *N* ammonium hydroxide using methyl red as an indicator, and adjusted to the desired concentration of ammonium chloride by addition of 1 *N* solution.

The electrolysis cell and dropping electrode were of the type described by Lingane and Laitinen (*9*). A saturated calomel electrode of large area was used as an anode. All potential measurements given below are referred to this electrode. The *m* and *t* values of the dropping electrode were, respectively, 1.931 mg. per second and 4.10 seconds with the capillary dipping into distilled water at 25° and an open electrical circuit. For most of the diffusion current readings, a Fischer Electropode with the galvanometer scale calibrated to read microamperes was used. For determining the equations of the rising portions of the curves and the half-wave potentials, a manual apparatus similar to that of Lingane and Kolthoff (*8*) was used to enable the potential to be determined with sufficient accuracy. All measurements were made with the electrolysis cell immersed in a water thermostat at 25°, regulated to ±0.02°.

Typical current-voltage curves for various concentrations of trivalent europium in 0.1 *N* ammonium chloride solution are shown in Figure 1. The values of the diffusion current, i_d, at −0.9 volt are given in Table I, together with the values corrected for the residual current. A satisfactory proportionality between the diffusion current and the europium concentration was observed.

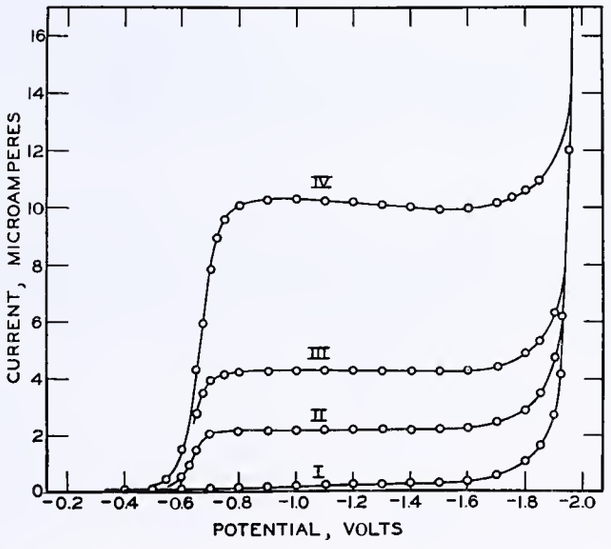

FIGURE 1. REDUCTION OF EUROPIUM IN 0.1 *N* AMMONIUM CHLORIDE

A millimolar solution of europium yielded a diffusion current of 2.88 microamperes. With the same dropping electrode, a millimolar solution of zinc chloride in 0.1 *N* ammonium chloride gave a corrected diffusion current of 6.34 microamperes. Taking into account the fact that the reduction of a zinc ion requires two electrons, it is evident that a 10 per cent correction is necessary if zinc is used as a standard in europium determinations.

It is apparent from Figure 1 that the diffusion current regions of europium, particularly with the highest europium concentration (curve IV), show a downward trend with increasing negative potential. This behavior, although de-

TABLE I. DIFFUSION CURRENT OF EUROPIUM IN 0.1 *N* AMMONIUM CHLORIDE

C	i_d Observed	i_d Corrected	i_d/C
Millimoles/liter	*Microamperes*		*Microampere/millimole/liter*
0.693	2.20	2.01	2.90
1.386	4.19	4.00	2.89
3.466	10.25	10.06	2.90
6.932[a]	19.75	19.56	2.83
			Av. 2.88

[a] In 0.2 *N* NH_4Cl.

scribed as anomalous by Holleck (*4*), is to be expected from the Ilkovic (*5*) equation for the diffusion current

$$i_d = 0.63\ nFCD^{1/2}m^{2/3}t^{1/6} \qquad (1)$$

The decreasing value of the drop time, t, with increasing negative potential causes a decreasing diffusion current. The total observed current is the sum of the residual current (curve I) and the true diffusion current. Because of the upward slope of the residual current line, the observed current increases continually with very low concentrations of europium but decreases with increasing negative potential at higher concentrations. The following data show that the effect is quantitatively predicted by Equation 1.

At a potential of −0.9 volt, the diffusion current, corrected for residual current, is 10.06 microamperes, while at a potential of −1.6 volts it is 9.53 microamperes. In this interval of potential the drop time changed from 4.06 to 2.98 seconds, while m remained practically constant. Multiplying the current of 9.53 microamperes by the factor $(4.06/2.98)^{1/6}$, we have 10.04 microamperes, which is in excellent agreement with the observed value at −0.9 volt.

Analysis of Europium Wave. It can easily be shown (*2*, *7*, *13*) that if the reduction of trivalent to divalent europium is reversible, the equation of the rising portion of the current-voltage curve should be given at 25° by

$$\pi = \pi_{1/2} + 0.059 \log \frac{i_d - i}{i} \qquad (2)$$

where π is the potential of the dropping electrode and i is the current at any point on the curve. The half-wave potential, $\pi_{1/2}$, should be independent of the europium concentration, and the slope of the straight line obtained by plotting log $\frac{i_d - i}{i}$ against the potential should be 0.059 volt if the reduction is reversible. A typical logarithmic plot for a europium wave is shown in Figure 2. The half-wave potential is given by the potential at which log $(i_d - i)/i$ is zero, and was found to be −0.671 ± 0.002 volt (*vs.* saturated calomel electrode) for various concentrations of europium or −0.425 volt referred to the normal hydrogen electrode.

The average slope of the logarithmic plot was found to be 0.079 volt rather than 0.059 volt as given by Equation 2, indicating that the dropping electrode does not behave as a strictly reversible europic-europous ion electrode. However, the deviation from reversible behavior is only small, as can be seen by a comparison of the half-wave potential with the normal potential of the europic-europous ion electrode.

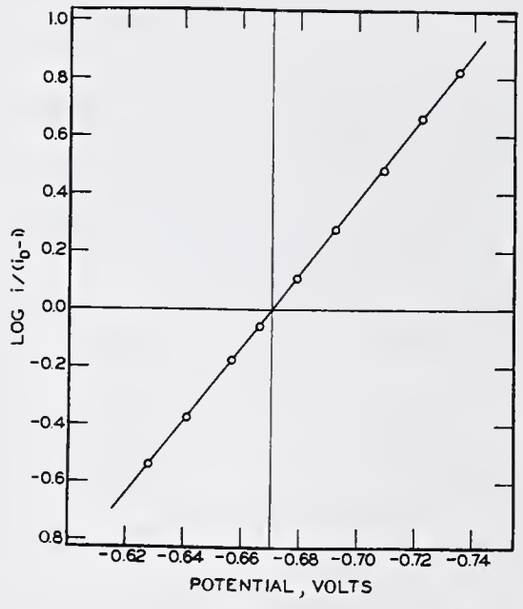

Figure 2. Analysis of Europium Reduction Curve

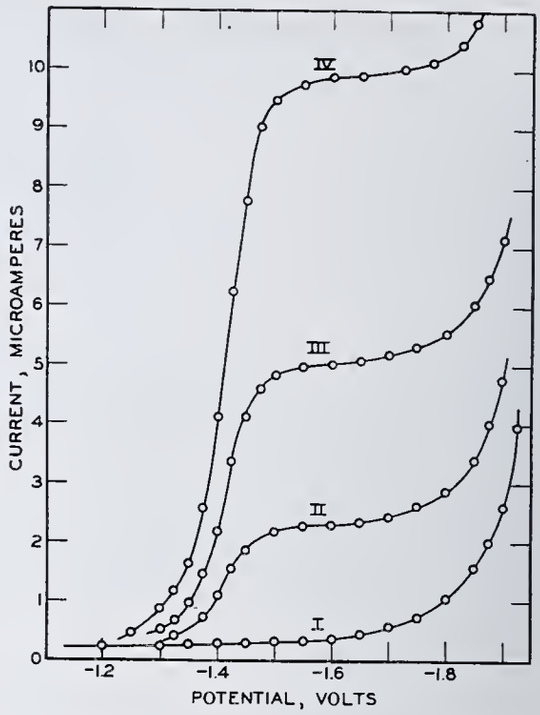

Figure 3. Reduction of Ytterbium in 0.1 *N* Ammonium Chloride

It can easily be shown that the half-wave potential and the normal potential, $\pi°$, are related by Equation 3 (assuming reversible electrode behavior).

$$\pi_{1/2} = \pi° + RT/F\ ln \frac{\gamma_{III}D^{1/2}_{II}}{\gamma_{II}D^{1/2}_{III}} \qquad (3)$$

γ_{III} and D_{III} are, respectively, the activity coefficient and diffusion coefficient of the trivalent ion and γ_{II} and D_{II} are the same quantities for the divalent ion. Although the values of γ and D for these ions are not known, it is reasonable to assume that $\gamma_{II} > \gamma_{III}$. Also $D_{II} > D_{III}$ because of the higher hydration of the trivalent ion. Thus the last term of Equation 3 cannot be large, and the half-wave potential should closely approach the normal potential if the electrode behavior is reversible. Actually the value of −0.425 volt for the half-wave potential is in close agreement with the value −0.43 volt for the normal potential (based on concentrations rather than activities) reported by McCoy (*11*).

Current-Voltage Curves of Ytterbium

A sample of ytterbium oxide prepared by a threefold electrolytic reduction of a rare earth fraction high in ytterbium content was available. Spectroscopic analysis showed traces of thulium and lutecium. The sample was dissolved in hydrochloric acid and treated in the same way as the europium sample.

Typical current-voltage curves for various concentrations of ytterbium in 0.1 *N* ammonium chloride are shown in Figure 3. The diffusion current values at −1.6 volts are given in Table II. By logarithmic plots similar to that shown in Figure 2, the half-wave potential was found to be −1.415 ± 0.003 volts (*vs.* saturated calomel electrode) or −1.169 volts (*vs.* normal hydrogen electrode). The average slope of the logarithmic plots was found to be 0.066 volt.

Table II. Diffusion Current of Ytterbium in 0.1 N Ammonium Chloride

C Yb^{+++}	i_d Observed	i_d Corrected	i_d/C
Millimoles/liter	*Microamperes*		*Microamperes/millimole/liter*
0.623	2.32	1.93	3.10
1.559	5.21	4.82	3.09
2.595	8.32	7.93	3.06
3.110	9.89	9.50	3.06
		Av.	3.08

Comparison of Diffusion Currents of Europium and Ytterbium

Owing to the closely similar structure of the various trivalent rare earth ions, and particularly the nearly identical conductance values of rare earth salt solutions (*6*), a much closer agreement would be expected between the diffusion current constants of europium and ytterbium than is shown by Tables I and II. In order to make an exact comparison it is necessary to correct the observed values to the same value of the capillary constant, $m^{2/3}\,t^{1/6}$. Correcting the observed value of 2.88 microamperes per millimole per liter for europium to the diffusion current region of ytterbium at -1.6 volts, we have 2.74 microamperes per millimole per liter. If the observed difference between europium and ytterbium were entirely due to a difference in the diffusion coefficient, a simple calculation from Equation 1 shows that the diffusion coefficients of ytterbium and europium would be in the ratio of 1.26 to 1.

It is probable, however, that the diffusion coefficients of europium and ytterbium ions are nearly equal and that another explanation accounts for the inequality of the diffusion currents. It is well known that ytterbous ions have a pronounced tendency to react with hydrogen ions according to the equation

$$2Yb^{++} + 2H^{+} \rightarrow 2Yb^{+++} + H_2 \qquad (4)$$

while europous ions have a much smaller tendency to undergo a similar reaction in a medium of the same pH. Thus the diffusion current value of ytterbium would be increased because of the increased supply of ytterbic ions at the electrode surface due to the reaction represented by Equation 4. If this view is correct, a slightly increased diffusion current of europium would be expected in a very strongly acid medium, in which the rate of oxidation of europous ions by hydrogen ions is increased. Actually, in 1.2 N hydrochloric acid solution a 5 per cent increase in the diffusion current of europium was observed. At higher acid concentrations no further increase was obtained, but a shift of the half-wave potential of europium to more negative values indicated a formation of complexes of europium with a large excess of chloride, with a consequent change of the diffusion coefficient. A similar effect could not be directly determined in the case of ytterbium because of interfering hydrogen-ion discharge even in very slightly acid medium. However, even in the presence of 10^{-4} N hydrochloric acid added to 0.1 N ammonium chloride, the observed diffusion current of 1.5×10^{-3} M ytterbium increased 7 per cent, partly because of hydrogen-ion discharge. Therefore great care must be taken to neutralize the excess acid used in dissolving ytterbium samples.

Determination of Europium

Since europium occurs only in very small concentrations in natural rare earth minerals, the usual analytical problem involves its determination in rare earth mixtures in which the europium concentration has been greatly increased by partial separation. In the present study, a synthetic mixture of europium and ytterbium oxides was analyzed for europium by the polarographic method and by titration after reduction with the Jones reductor.

Procedure. For the polarographic analysis, a weighed sample was dissolved in hydrochloric acid and treated as described above. The final electrolysis was carried out in a medium of 0.1 N ammonium chloride. From the diffusion current value at a potential of -0.9 volt, the percentage of europium was calculated, using the calibration value from Table I. A current voltage curve of 25 ml. of solution containing 0.00995 gram of rare earth as oxide is shown in Figure 4.

The volumetric determination used was a modification of the method of McCoy (*11*), in which the reduced europium solution is passed into a standard iodine solution and back-titrated with standard thiosulfate. Difficulties due to volatilization of iodine were encountered, with the result that the determinations were not uniformly reproducible. However; the most closely checking values by the method of McCoy were in agreement with those given below. Substitutions of stannic chloride for iodine removed the possibility of error due to volatilization and yielded the advantage of a direct titration over a back-titration. Forty-five milliliters of a solution containing 0.3498 gram of rare earth as oxide and 0.2 N in hydrochloric acid were passed through a Jones reductor into 30 ml. of 0.5 N stannic chloride solution. The reducing column was washed with 100 ml. of 0.06 N hydrochloric acid. Each 100 ml. of solution was treated with 20 ml. of concentrated hydrochloric acid and 2 ml. of 0.02 per cent solution of diphenylamine in concentrated sulfuric acid. The resulting solution was titrated with standard 0.1 N potassium dichromate. An atmosphere of nitrogen was used throughout the reducing column and the titrating vessel. Although the titration reaction was slow near the end point, a definite end point yielding reproducible results was observed.

Results. Two polarographic determinations with widely differing rare earth concentrations gave results of 55.0 and 54.7 per cent of europium oxide, respectively. Two volumetric determinations yielded 56.6 and 56.5 per cent of europium oxide, respectively.

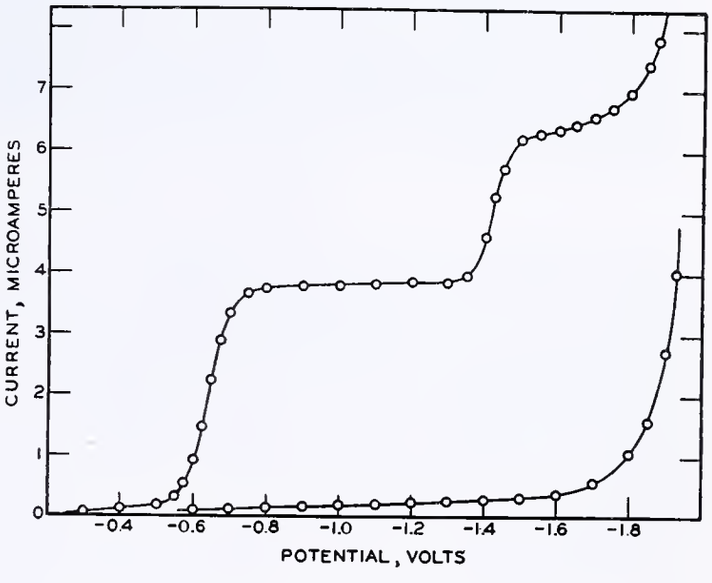

Figure 4. Current-Voltage Curve of Europium-Ytterbium Mixture

The results obtained by the two methods agree to an accuracy of 3 per cent. The polarographic procedure has the advantages of greater speed and simplicity and is applicable to samples containing only traces of europium. The volumetric method is probably more accurate with samples of high europium content, but requires much larger samples and involves a much more difficult recovery procedure for the rare earth material.

Determination of Ytterbium

The successful polarographic determination of ytterbium in rare earth mixtures depends upon obtaining well-defined

Table III. Composition of Ores

Spectroscopic Composition, %	I Euxenite	II Fergusonite	III Samarskite	IV Xenotime	V Cerite
Y	29.0	25.0	21.8	10.25	2.0
Nd	7.4	7.4	6.8	19.5	20.00
Sm	4.55	3.3	4.9	6.0	4.25
Gd	4.1	3.4	4.2	4.0	1.75
Dy	3.3	2.4	6.4	3.3	1.0
Yb	6.05	6.2	1.5	1.7	1.0
La	...	..	..	10.5	9.9
Polarographic Percentage					
Yb	5.3	7.0	0.5	...	...

reduction waves of ytterbium in the presence of relatively large concentrations of the other rare earths. Preliminary experiments showed that if the ytterbium concentration was 25 per cent or more of the total rare earth content, well-defined waves having the appearance of those shown in Figure 3 were obtained.

In order to determine its limits of applicability, the polarographic method was applied to the analysis of mixed rare earth oxides obtained from various representative classes of naturally occurring minerals. Oxide samples which had been prepared and analyzed spectroscopically by McCarty, Scribner, Lawrenz, and Hopkins (*10*) were available. The composition of the various ores, as determined spectroscopically, is given in Table III, together with the polarographic values for ytterbium.

Procedure. Approximately 2 grams of the oxide mixture were dissolved in 15 ml. of 6 *N* hydrochloric acid and made up to 100 ml. in a volumetric flask. A 10-ml. portion was pipetted into a 50-ml. volumetric flask. After the addition of a drop of methyl red solution, the sample was titrated with 0.5 *N* ammonium hydroxide, using a color comparison standard of 25 ml. of 0.1 *N* ammonium chloride containing a drop of methyl red. Sufficient 1 *N* ammonium chloride was added to bring its concentration to 0.1 *N* after dilution to 50 ml. The current-voltage curve was determined in the usual way.

Results. Samples I, II, and III (Table III) were found to yield well-defined ytterbium reduction waves. The diffusion current region was most nearly horizontal in the case of sample II, and had an increasing upward slope with samples I and III in that order. Table III shows that the spectroscopic value for the samarium content of these samples increases in the order of samples II < I < III. Since samarium is the next most easily reducible trivalent rare earth after ytterbium (*12*), it is probable that a relatively high samarium content can affect the imaginary residual current curve of the rare earth sample containing no ytterbium; hence it is impossible to determine the true residual current value, which would have to be subtracted from the measured ytterbium diffusion current in order to calculate the percentage of ytterbium. The usual polarographic procedure of determining the diffusion current by the vertical distance between parallel tangents to the current-voltage curve was therefore used. In the case of sample II, the tangent method yielded the same result as was obtained by subtracting the residual current of the 0.1 *N* ammonium chloride medium, while lower results were obtained by the tangent method than by the subtraction method for samples I and III where the diffusion current showed an abnormal upward slope.

The polarographic and spectroscopic values for samples I and II were found to check within the limits of accuracy of the latter method, which were stated to be ±15 per cent (*10*). For sample III, a lower value was obtained by the polarographic method.

To check the possibility of interference of other constituents of the sample, known amounts of ytterbium were added to solutions of samples II and III, and the increase in the diffusion current of ytterbium at a potential of −1.6 volts was determined.

Table IV gives the results of adding ytterbium, in the form of a solution made from a mixture of rare earth oxides containing 75.5 per cent ytterbium ozide, to 25 ml. of a solution of sample II containing 0.1160 gram of rare earth oxides. The amount of added ytterbium found was calculated from the increase in the diffusion current, allowing for dilution.

Table V gives the results of similar additions of ytterbium to 25 ml. of a solution of sample III, containing 0.0886 gram of rare earth oxides.

Tables IV and V indicate that no interfering substances are contained in the fergusonite sample, but that some constituent of the samarskite sample causes interference with the polarographic determination. The nature of the interfering substances is unknown because complete analyses of the mixtures were not available.

Samples IV and V showed no ytterbium reduction waves, because the final unlimited current rise began sooner than in the above cases. Apparently there is interference by some other constituents of the oxide mixture in these cases. The first three samples are yttrium earth ores, while the last two are relatively low in yttrium and high in cerium group content.

It is concluded that reliable polarographic determinations of ytterbium can be made in rare earth mixtures if the ytterbium reduction wave is well defined with a reasonably constant diffusion current region. This condition is favored by an increasing ytterbium content and decreasing samarium content, and appears to be fulfilled in the case of yttrium earth ores, but not in ores high in cerium group content. The polarographic method should be useful in following changes in ytterbium content in making rare earth separations.

Table IV. Addition of Ytterbium to Fergusonite

Increase in *id* *Microamperes*	Yb_2O_3 Added *Mg.*	Added Yb_2O_3 Found *Mg.*
0.53	0.79	0.88
1.33	2.37	2.39
2.08	3.95	3.99

Table V. Addition of Ytterbium to Samarskite

Increase in *id* *Microamperes*	Yb_2O_3 Added *Mg.*	Added Yb_2O_3 Found *Mg.*
0.35	0.79	0.59
1.07	2.37	1.92
1.71	3.95	3.30
2.58	6.32	5.48

Simultaneous Determination of Europium and Ytterbium

Since the reduction potentials of europium and ytterbium are widely separated, no difficulty is encountered in making a simultaneous determination of the two elements. However, in determining the diffusion current of ytterbium in such a mixture, care must be taken to correct for the decreasing diffusion current of europium with increasing negative potential (Figure 1).

The recommended procedure may be described by reference to Figure 4. The total diffusion current of ytterbium and europium at −1.6 volts is 6.00 microamperes after subtracting the residual current. The observed diffusion current of europium at −0.9 volt is 3.58 microamperes. Multiplying by

the factor $\left(\frac{2.98}{4.06}\right)^{1/6}$ as above, we find 3.40 microamperes as the diffusion current of europium at a potential of −1.6 volts. Thus the true diffusion current of ytterbium is 6.00 − 3.40 or 2.60 microamperes.

The results obtained for ytterbium, in the mixture of europium oxide and ytterbium oxide described above, were 42.0 and 43.1 per cent, respectively, in two determinations with different concentrations of rare earth. The summations of europium oxide and ytterbium oxide were 97.0 and 97.8 per cent, respectively. Since the sample was known to contain only small amounts of other rare earths, it is believed that the results for ytterbium are essentially correct. If no correction had been made for the effect of decreasing drop time on the europium diffusion currents, the summations would have been 94.1 and 94.7 per cent, respectively. The tangent method of measuring wave heights would likewise lead to erroneously low results for ytterbium because of the unsymmetrical nature of the second wave. The magnitude of the correction due to changes of drop time will, of course, increase with an increasing ratio of europium to ytterbium.

Summary

Ammonium chloride is a suitable indifferent electrolyte for the determination of europium and ytterbium. In 0.1 *N* ammonium chloride the half-wave potentials of europium and ytterbium were found to be −0.671 and −1.415 volts with reference to the saturated calomel electrode.

The polarographic method for europium gives results agreeing within 3 per cent with the volumetric method using the Jones reductor.

Conditions favoring the successful polarographic determination of ytterbium are discussed. Reliable results can be obtained if well-defined reduction waves resembling those of pure ytterbium are obtained. Satisfactory results were obtained with yttrium earth ores containing about 5 per cent ytterbium. Ores of low ytterbium content, particularly those high in cerium group content, were found to yield low results by the polarographic method.

In the simultaneous determination of europium and ytterbium it is necessary to correct for the effect of decreasing drop time with increasing negative potential.

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Determination of Lead Content of Commercial Ciders and Vinegars by Spectrographic Methods

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BECAUSE of the necessary spraying of vegetation subsequently to be used for food there results a certain amount of residual lead which often cannot be removed, particularly in the by-products of the apple crop. Excessive spray residue is often removed from apples which are sold for consumption as such, but not necessarily from apples intended for use in the manufacture of cider and vinegar. This investigation was undertaken to devise rapid and accurate spectrographic methods for the determination of lead in cider and vinegar, as well as to determine the range of lead content of these products as they appear on the market.

All apparatus used was freed from lead by rinsing with hot nitric acid and lead-free water redistilled in a Pyrex still. The bismuth chloride was freed from lead by precipitating bismuth oxychloride with lead-free water, decanting the supernatant liquid, dissolving the precipitate in double-distilled hydrochloric acid, and repeating the process until the salt was spectroscopically free from lead. The calcium acetate was freed from lead by precipitating with hydrogen sulfide, using copper as a coprecipitant.

Analysis of Cider. Twenty-five cubic centimeters of cider were placed in a platinum dish, to which were added 0.5 mg. of bismuth as bismuth chloride and 0.04 gram of calcium acetate. The addition of calcium was found to enhance both the lead and the bismuth lines. The mixture was evaporated nearly to dryness on the steam bath and charred over a small flame, being allowed to take fire when charring was almost complete. The char was ground to a fine powder and three approximately equal portions of such size, experimentally determined, as would give lines of convenient length on the plate were placed in cupped graphite electrodes—about one sixth of the total char was placed in each electrode. It was found unnecessary to weigh these portions, as considerable variation in the amount used had no appreciable effect on the analysis. These samples were then burned in an arc of 220 volts and 9 amperes, the lead being determined by the internal standard method, using a rotating logarithmic sector in front of the slit. The lengths of the bismuth line at 2898 Å. and the lead line at 2833 Å. were compared. The lengths of the lines from the divided samples were averaged before plotting.

Table I. Lead Content of Cider and of Vinegar

	Cider	Cider Vinegar	Distilled or Spirit Vinegar	Malt Vinegar	Malt and Spirit Vinegar
No. of samples	13[a]	49	5	1	1
	Parts per million				
Lowest	0.10	0.18	0.00	..	..
Lower quartile	0.18	0.37	..	..	..
Median	0.27	0.51	..	..	..
Average	0.43	0.55[b]	0.26	0.03	0.08
Geometric mean	0.32	0.50[b]	..	..	..
Upper quartile	0.54	0.78	..	..	..
Highest	1.50	11.80[c]	1.20	..	..

[a] Not including 5 samples containing less than 1 part lead per million.
[b] Exclusive of highest sample. Including highest sample: average = 0.78 geometric mean = 0.53.
[c] Duplicate determination by dithizone extraction method gave 8.0 parts of lead per million.

In a series of seven analyses in the range of from 0.14 to 3.4 parts per million of lead the average error was 1.5 per cent and the maximum error was 6.0 per cent, using cider containing known added amounts of lead. In this case six separate differences in line lengths were averaged to give each of the seven points on the working curve. Eighteen samples of commercial cider were examined, and all contained minute amounts of lead (Table I). Five samples which were examined only to an accuracy of less than 1 p. p. m. of lead are not included in the table.

Analysis of Vinegar. Twenty-five cubic centimeters of vinegar were evaporated to dryness in a platinum dish, and the residue was dissolved in 0.5 cc. of double-distilled hydrochloric acid containing 1 mg. of bismuth per cc. The well-mixed solution of the vinegar residue was divided equally among three cupped graphite electrodes which had previously been treated with one drop of lead-free kerosene to prevent the solution from soaking into the electrodes. The determination was then carried out as in the cider method.

In one series of twelve analyses, using vinegar containing known added amounts of lead in the range of from 0.4 to 1.2 p. p. m., the average error was 7 per cent and the maximum error was 20 per cent. In another series of six analyses in the range of from 0.08 to 0.4 p. p. m. of lead the average error was 9 per cent and the maximum error was 25 per cent.

Fifty-six samples of commercial vinegar were examined, and all but two, both distilled and spirit vinegar, contained lead (Table I). The geometric mean is nearer the median than is the arithmetic mean, indicating the geometric nature of the series.

The lead tolerance adopted by the United States Department of Agriculture under the old food and drug law was 0.025 grain per pound, which is equivalent to 3.5 p. p. m. Only one of these samples exceeded that figure. The next lower result was 1.8 p. p. m. or approximately one half of the tolerance.

The lead content of the cider was on the average less than that of the cider vinegar. There are two possible explanations. The cider was made from New England apples which do not require so much spraying as do western apples, but the vinegar was not exclusively a Massachusetts product. It is customary in the manufacture of sweet cider prior to placing it on the market to filter the apple juice through sand or some other type of filter which may remove some of the lead, but in the manufacture of vinegar this filtration is not always carried out. Furthermore, vinegar is a good solvent for lead in paint, metals, etc., with which it may come in contact during processing.

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A Method for the Identification of Nitriles

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SEVERAL methods have been proposed for the identification of nitriles (*1*, *2*, *3*). The authors have found that adaptation of the well-known method of reduction of nitriles to primary amines by sodium and absolute alcohol gives a practical method for the identification of aliphatic nitriles. Aromatic nitriles do not give such good results, but this class can usually be readily determined by hydrolysis to the corresponding amide or acid.

Procedure

A solution of 1 cc. (0.8 to 1.0 gram) of the nitrile in 20 cc. of absolute alcohol is placed in a 200-cc. round-bottomed flask fitted with a reflux condenser. (It is essential that the alcohol be absolute, otherwise considerably less derivative is obtained.) The flask is immersed to the neck in a water bath heated to 50° to 60° C. Fresh, finely cut sodium (1.5 grams) is added gradually through the top of the condenser as rapidly as possible without allowing the reaction to become too vigorous. When all the sodium has reacted (10 to 15 minutes) the reduction is complete.

The mixture is cooled to 20°, and 10 cc. of concentrated hydrochloric acid are added in small portions through the top of the condenser. Care is necessary on account of the spattering which takes place when the acid strikes the strongly alkaline mixture.

The reflux condenser is disconnected, the system is set up for ordinary distillation, and 20 cc. of alcohol are distilled into a graduated cylinder. The residue in the flask is cooled to 20° and a solution of 6 grams of sodium hydroxide in 6 cc. of water is cautiously added. The reaction at this point is violent, and care is necessary to avoid loss of amine by volatilization. The flask is swirled to ensure mixture of the ingredients and then rapidly reconnected to the condenser. Using a smoky flame the flask is heated until the contents are nearly dry, catching the distillate in a 50-cc. Erlenmeyer flask containing 3 cc. of water. The condenser should be fitted with an adapter dipping beneath the surface of the water in the flask.

If the original substance was a nitrile, the distillate will be alkaline at this point.

Phenylisothiocyanate (0.5 to 1.0 cc.) is then added to the distillate, and the mixture vigorously shaken for 2 or 3 minutes. If no derivative forms on shaking, scratching the walls of the Erlenmeyer and cooling under a tap or in an ice bath will bring down the precipitate. Aliphatic derivatives as a rule respond to shaking; aromatic compounds require cooling in an ice bath. The crude derivative is filtered, washed with 50 per cent alcohol and recrystallized from dilute alcohol in the usual manner.

Because reduction in the case of aromatic nitriles is less smooth, an initial sample of 2 cc. or 2 grams is recommended.

In Table I are given the results obtained with ten aliphatic and four aromatic nitriles. The product was in most cases recrystallized from dilute alcohol, two recrystallizations usually being sufficient to yield a pure product. The method is applicable only to those aliphatic nitriles which form a volatile amine upon reduction. In the aromatic series the method works less well, probably because of the lack of volatility of the amine and the fact that reduction in the aromatic series is accompanied by side reactions.

Table I. Identification of Nitriles

	Weight of Nitrile Used	No. of Crystallizations	M. P. of Phenyl Thiourea	Weight of Derivative Obtained	Nitrogen Calculated	Nitrogen Found
			° C.	Gram	%	%
Acetonitrile	0.8	2	106	0.8	...	...
Propionitrile	0.8	2	63	0.8	...	...
n-Butyronitrile	0.8	2	65	0.7	...	...
Isobutyronitrile	0.8	2	82	0.6	...	...
n-Valeronitrile	0.8	2	69	0.7	...	...
Isovaleronitrile	0.8	2	102	0.6	...	...
n-Capronitrile	0.8	2	77	0.3	...	...
Isocapronitrile[a]	0.8	2	112	0.25	11.85	11.97
Glutaronitrile[a]	0.8	2	148	0.9	15.04	15.00
Succinonitrile[a]	0.7	2	168	0.9	15.63	15.64
Benzonitrile[a]	2.0	2	147	0.3	11.56	11.62
p-Tolunitrile[a]	1.0	3	144	0.10	10.93	10.84
o-Tolunitrile[a]	3.0	4	179	0.3	10.93	11.35
β-Naphthonitrile[a]	3.0	5	140	0.33	9.58	9.69

[a] Derivatives not previously described. No satisfactory results could be obtained with α-naphthonitrile or with *m*-tolunitrile.

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Continuous-Reading Electronic Voltmeter

For Use with Glass and Other High-Resistance Electrode Systems

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A full-range, sensitive, low-current-drain, continuous-reading electronic voltmeter is described. The meter has a range of ±2.11 volts and a sensitivity of ±0.001 volt over the entire range. It has been designed for the accurate determination of potentials of electrode systems that have a resistance of 5000 megohms or less.

THE increased use of durable glass electrodes and nonaqueous, high-resistance titration media in potentiometric titrations has emphasized the need for a versatile potentiometer, or millivoltmeter, that will satisfactorily measure the potential between electrodes of a cell having a resistance of 150 to 5000 megohms between the electrode terminals. Instead of the combination of potentiometer, thermionic galvanometer, and electrode system entirely surrounded by a large electrostatic shield through which the adjustments are made by remote control, the authors have found it advantageous to use a continuous direct-reading meter along with flexible shielded leads and electrodes. [The Beckman pH meter, Industrial Model M, and the Beckman Model O electronic voltmeter (National Technical Laboratories, Pasadena, Calif.) are commercial examples. The latter is a versatile meter with operating characteristics equivalent, except for somewhat lower sensitivity, to those of the meter described in this paper.] The continuous indicating meter not only requires less manipulation by the operator, but also enables him easily and definitely to ascertain when the potentials have reached equilibrium. For greatest usefulness and versatility, a modern meter should continuously indicate the applied e. m. f., operate on less than 10^{-12} ampere over the entire range of approximately 2 volts, be sensitive and accurate to ±0.001 volt in any part of the range, and provide a simple means of attaching the e. m. f. source to the meter by means of flexible shielded leads (*14*).

Since the first application of the thermionic electron tube by Goode in an electro-titration meter (*7*), recognition of the

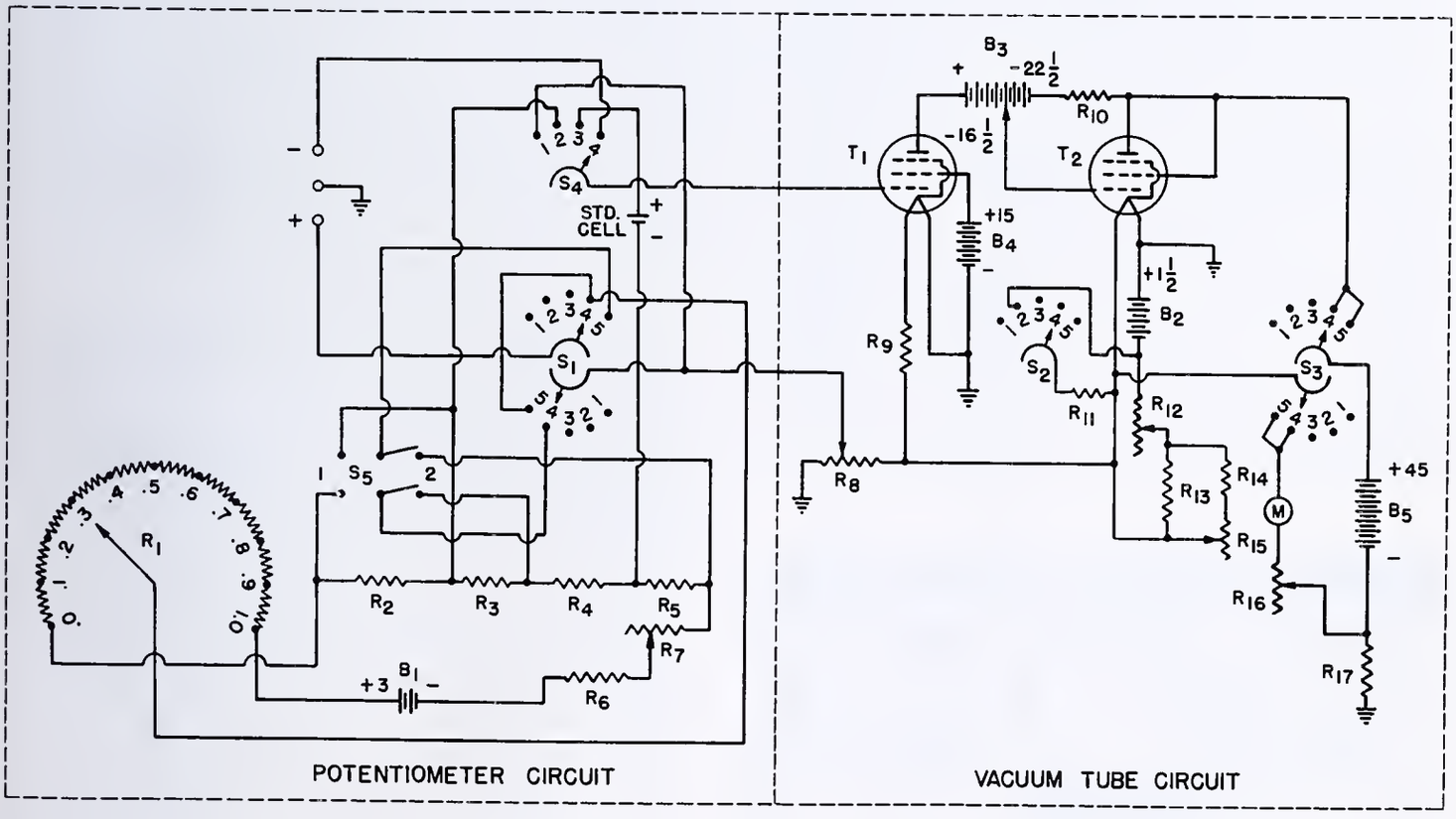

FIGURE 1. DIAGRAM OF CIRCUIT

M. Weston Model 801 0–0.20 direct current milliammeter (400 ohms)
R_1. General Radio Type 510, decade resistance 100 ohms per step
R_2. 110-ohm precision resistor
R_3. 890-ohm precision resistor
R_4. 19.0-ohm precision resistor
R_5. 91.0-ohm precision resistor
R_6. 400-ohm wire-wound
R_7. 600-ohm wire-wound variable
R_8. 1000-ohm wire-wound variable
R_9. 7.5 ohms
R_{10}. 10 megohms
R_{11}. 25 ohms
R_{12}. 6-ohm wire-wound with "off" position
R_{13}. 1-ohm wire-wound
R_{14}. 5-ohm wire-wound
R_{15}. 25-ohm wire-wound variable
R_{16}. 15,000-ohm wire-wound variable
R_{17}. 2000 ohms
S_1. 5-position 2-circuit Centralab selector switch
S_2. 5-position single-circuit shorting type Centralab
S_3. 5-position 2-circuit Centralab selector switch
S_1, S_2, and S_3 mounted on same shaft operated by a single control
S_4. Centralab Isolantite selector switch
S_5. Double-pole double-throw jack switch
Std. Cell. Eppley saturated 1.0190 volts
T_1. Type 32
T_2. Type 1A5G

importance of electronic millivoltmeters in analytical chemistry has progressed steadily (*1–13, 15–20*). These titrimeters require a constant filament current; for this reason battery-operated meters often have been preferred to line-operated alternating current equipment. Most of these meters have been found objectionable from the standpoint of application to high-resistance systems (100 to 2000 megohms), because of lack of sensitivity over their entire range, inadequate shielding of electrode leads, or the presence of a grid current greater than 10^{-11} ampere. The object in developing the electronic voltmeter described in this paper was to obtain a universal meter particularly suited for analytical laboratory use.

Electrical Circuit

Since it is practically impossible to meet the requirement of wide range with high sensitivity in a single indicating meter, a step potentiometer has been combined with an electronic voltmeter; the potentiometer has ten steps of 0.100 volt each and one step of 1.000 volt. The indicating meter has a range of 0.110 volt, so that the total range is ±2.110 volts. The smallest scale division is 0.002 volt, and it is easily possible to estimate the reading to 0.0005 volt.

The circuit is shown in Figure 1. The vacuum tube circuit is that of a two-stage direct-coupled amplifier using selected, aged, standard receiver type vacuum tubes. The first tube (Type 32) is used with reduced potentials on all elements to reduce primary and secondary emission from the elements, is covered with a coating of ceresin wax to decrease surface leakage, and is shielded from light to prevent photoemission from the grid. The tube must be tested for microphonic properties and grid current under operating conditions of mounting, etc. (Tested tubes are obtainable from the National Technical Laboratories.)

The filament resistor network (R_9, R_{12}, R_{13}, R_{14}, R_{15}) serves as a coarse and vernier control for accurately adjusting the joint filament current of both tubes when making the zero adjustment of the circuit. For the full-scale adjustment, the selector switch, S_4, places a negative 0.110 volt across the input and requires adjustment of resistor R_{16} for full-scale meter deflection. It is apparent that there is interlocking between the two control adjustments that necessitates one or two readjustments of each control until both ends of the scale are correct. The reversing switch, S_1, position 5, applies an additional −0.110 volt between the electrode terminals, so that the meter deflects full scale when the externally applied e. m. f. is 0 volt; the reverse scale on the meter is used for this setting of switch S_1. With this setting the potentiometer leads are reversed and the voltmeter leads remain unchanged. The meter indicates an up-scale deflection when a more negative potential is applied between the electrode terminals. With S_4 in position 3, the potentiometer circuit is standardized against the standard cell potential in the usual manner, using R_7 for adjustment and the vacuum tube meter for the null indicator. In this operation, the unbalanced potential between the standard cell voltage on the potentiometer and the actual voltage of the standard cell is placed across the terminals of the voltmeter. Adjustment R_8 is used to set the grid bias voltage to a point which gives the most linear calibration. In some instances, the linear response may be improved by varying the 15-volt screen potential and the load resistor, R_{10}.

By turning resistor R_{12} to off position and selector switch S_2 to position 2, the meter is on the standby position in which the filaments are operated at one-half operating current and the plate battery is disconnected. The meter is turned off by setting R_{12} on the off position and placing S_2 in position 1. In order to avoid erratic changes in the meter adjustment due to small changes in contact resistance of the switch, a separate switch is not used in the filament circuit. For the same reason, it is recommended that the two rheostats used in the filament circuit (R_{12} and R_{15}) should have a spiral "pigtail" connection between the rotor and the variable terminal.

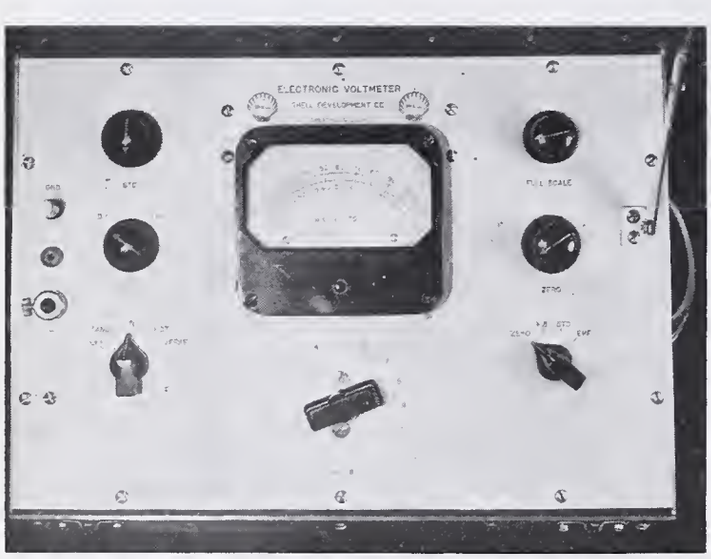

Figure 2. Instrument Panel

Constructional Details

The unit is housed in a metal cabinet and a sloping control panel is provided to facilitate observation of the indicating meter and adjustment of the controls. It is essential to surround the entire unit with metal cabinet, or metal-lined cabinet, in order to avoid disturbing influence of stray external potentials. The control panel is arranged for convenience of operation; the arrangement of the control knobs is shown in Figure 2. Multipoint switches are used for direct and reverse electrode connections, plate battery and standby connections, and standardizing operations. Adjustable controls are provided for zero, full-scale, and potentiometer standardizing adjustments.

The rugged d'Arsonval-type indicating meter is calibrated linearly from 0 to 110 millivolts and from 110 to 0 millivolts. The "reverse" scale is provided so that it is not necessary to subtract the meter reading from the potentiometer setting when the electrode terminals are connected in the reverse position.

In order that the instrument may function on a high input resistance, good proved insulation is used throughout the grid circuit of the first tube. Switch S_4 is made of Isolantite and is coated with ceresin wax. The wire between S_4 and the grid cap, and the wire between the inlet jack and S_4, are exceedingly well insulated by using the best grade of stiff insulated wire and installing the wire so that it is air-insulated throughout. The shielded inlet terminal is shown in Figure 3; it is similar to the one used on the Beckman pH meters and will accommodate the plug used on the Beckman shielded glass electrode. Battery B_3 is insulated from the housing and other batteries by separators made of thin Bakelite. Battery B_2 is made up of four or more Burgess Type 4FJ batteries connected in parallel.

Operation Procedure

Set the multiple selector switch, S_1, S_2, and S_3, on position 4 (Direct) and switch S_4 on position 1 (Zero). Vary R_{15} (Zero adjustment) and R_{12} if necessary (not shown on panel) until the meter reads up scale and finally returns to exactly zero reading as the filament resistance decreases. Set S_4 on position 3 (STD) and adjust the standardizing rheostat, R_7, until the meter needle indicates zero reading. Turn S_4 to position 2 (F. S.) and vary R_{16} (Full-Scale adjustment) until the meter indicates exactly 110 units. Repeat these adjustments until they can be readily duplicated. Connect the electrode terminals to the e. m. f. source, set S_4 to position 4 (E. M. F.), and note the position of the indicating needle. If it indicates more than full scale, leave the multiple selector switch, S_1, S_2, and S_3, on position 4 (Direct) and vary selector R_1 and switch S_5 (Volts adjustments) until the meter indicates on scale. The reading indicated by the position of R_1 and S_5, plus that of the direct scale on the indicating meter, indicates the positive voltage between the terminals. If the needle indicates off scale below zero, turn S_1, S_2, and S_3 to position 5 (Reverse) and manipulate R_1 and S_5 until the needle comes on scale. The sum of the reading indicated by the position of R_1 and S_5 and the reverse scale on the meter indicates the voltage between the terminals (of opposite polarity to that indicated).

The meter, cell stand, motor frame, and other accessories are connected to a ground in order to avoid errors due to stray electrostatic potentials and alternating current leaks.

When the meter is used daily, the multiple selector switch, S_1, S_2, and S_3 is turned to position 3 (on) and S_4 is turned to position 1 (Zero) between determinations and overnight. For intermittent use, the filament adjusting rheostat, R_{12}, is turned to the off position and the multiple selector switch, S_1, S_2, and S_3, is placed on position 2 (Standby), and the meter is adjusted to operate approximately one hour before it is used. By maintaining the instrument in this condition, when it is not used regularly, the drift and battery consumption are reduced to a minimum. The me-

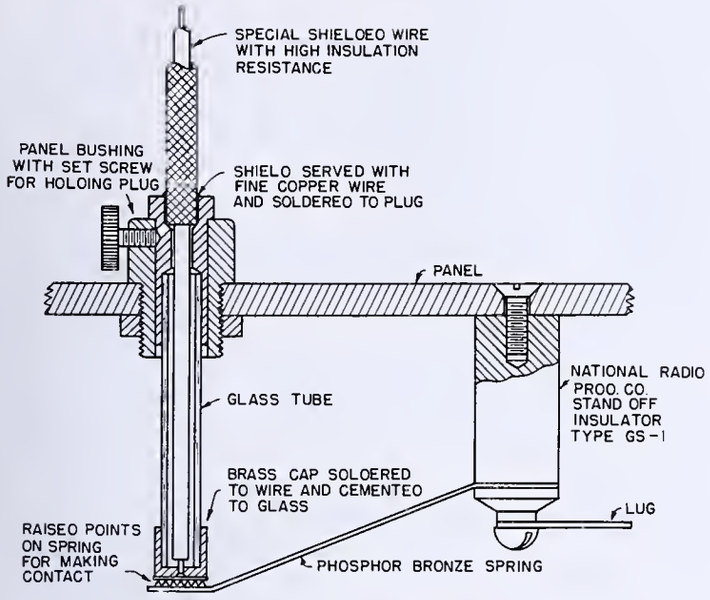
SPECIAL SHIELDED WIRE WITH HIGH INSULATION RESISTANCE
PANEL BUSHING WITH SET SCREW FOR HOLDING PLUG
SHIELD SERVED WITH FINE COPPER WIRE AND SOLDERED TO PLUG
PANEL
NATIONAL RADIO PROD. CO. STAND OFF INSULATOR TYPE GS-1
GLASS TUBE
BRASS CAP SOLDERED TO WIRE AND CEMENTED TO GLASS
LUG
RAISED POINTS ON SPRING FOR MAKING CONTACT
PHOSPHOR BRONZE SPRING

FIGURE 3. SHIELDED INLET TERMINAL JACK

ter is completely turned off by putting S_1, S_2, and S_3 on position 1 and turning the filament adjusting rheostat, R_{12}, to its off position.

Operating Characteristics

The meter is capable of continuously measuring the potential across electrodes of any system that has a resistance less than 5000 megohms with a precision of ±0.001 volt. The systematic error is less than ±0.005 volt using a linear indicating meter scale and is less than ±0.001 volt using a calibrated scale. Potential differences of ±2.11 volts may be measured with a maximum current drain of approximately 3×10^{-13} ampere. The meter is especially useful in adjusting a system to a definite potential difference, in observing the change of potential over a period of time, and in making all kinds of electrometric titrations. The meter may safely be used to measure any potential without previous knowledge of the magnitude or sign of the potential being measured, and without danger of polarization of the electrode system.

A typical example of the influence of the input resistance on the accuracy of the meter is given in Table I. The deviations at zero input resistance indicate the slight nonlinearity of the instrument under test; the deviations at 5000 megohms are hardly greater, indicating that the error introduced by this resistance is negligible. The error increases for greater input resistances, but is of such nature and magnitude that the meter operates satisfactorily as a titration meter even with input resistances as high as 100,000 megohms.

In any vacuum tube circuit capable of functioning on such high input resistance, the problem of drift becomes important and is usually considered to be exceedingly difficult to solve. While drift has not been completely eliminated in this circuit, it has been reduced to 0.002 volt per hour; this is not excessive and allows measurements of the desired accuracy with only occasional resetting of the zero adjustment. This value of drift is obtained only after the meter has been in operation for several hours. If the meter has been left on standby for some time, the drift is approximately 0.020 volt the first hour, after which it rapidly diminishes to the regular value. The potentiometer circuit and the full-scale adjustment do not drift more than 0.001 volt in 8 hours unless the batteries have been depleted.

The four filament supply batteries will operate the meter continuously for 4 months. The life of the 45-volt (B_5) battery is approximately 6 months; it must be replaced when its voltage falls below 40 volts. The remainder of the batteries have approximately "shelf life", as the current drains are negligible.

Discussion

The vacuum tube circuit is a modification of a similar previous circuit using Type 1B4 (tetrode) and 1F4 tubes in place of Type 32 and 1A5G, respectively. In the early circuit, 3-volt filament supply batteries and a 0 to 1.0-milliammeter were used along with the following changes: $B_4 = 13.5$ volts; $B_2 = 3$ volts; $R_{10} = 7$ megohms; $R_{16} = 600$ ohms; $R_9 = 34$ ohms; and $R_{12} = 10$ ohms. The earlier circuit operated with a grid current drain of less than 5×10^{-12} ampere, and for systems having a resistance less than 500 megohms was capable of exactly linear calibration and accuracy within 0.0005 volt. However, for input resistances over 1000 megohms, the modified circuit showed an advantage in accuracy; thus at 11,500 megohms it had about one quarter the error of the earlier circuit. Some trouble was experienced in selecting for the earlier circuit Type 1B4 tubes that were not subject to microphonic disturbances and were insulated well enough to give satisfactorily low grid current drain.

A possible improvement would be to enclose all the batteries in a separate compartment, leaving the tubes and other components in a practically gas-tight space. This arrangement would prevent the ammonia fumes and moisture released by the batteries from creating leakage paths over insulated surfaces and would allow the use of a dehydrator in the tube compartment. However, a properly installed tube and accessories will not exhibit troublesome surface leakage, provided the cabinet contains several small vents for air circulation.

TABLE I. INFLUENCE OF INPUT RESISTANCE ON METER READING

Applied E. M. F. *Volts*	Meter Reading on Linear Scale *Volts*	Deviation from Applied Value *Volts*	Input Resistance *Megohms*
0.0	0.000	0.000	0.0
0.012	0.010	0.002	0.0
0.022	0.020	0.002	0.0
0.033	0.030	0.003	0.0
0.043	0.040	0.003	0.0
0.052	0.050	0.002	0.0
0.062	0.060	0.002	0.0
0.072	0.070	0.002	0.0
0.081	0.080	0.001	0.0
0.090	0.090	0.000	0.0
0.100	0.100	0.000	0.0
0.110	0.110	0.000	0.0
0.0	0.000	0.000	5,000
0.053	0.050	0.003	5,000
0.101	0.100	0.001	5,000
0.111	0.110	0.001	5,000
0.003	0.000	0.003	11,500
0.055	0.050	0.005	11,500
0.102	0.100	0.002	11,500
0.115	0.110	0.005	11,500
0.014	0.000	0.014	50,000
0.066	0.050	0.016	50,000
0.116	0.100	0.016	50,000
0.128	0.110	0.018	50,000
0.024	0.000	0.024	110,000
0.081	0.050	0.031	110,000
0.133	0.100	0.033	110,000
0.146	0.110	0.036	110,000
1.146[a]	1.110[a]	0.036	110,000

[a] Higher e. m. f.'s (introduced by step potentiometer) cause no further error, as may be seen by comparison with figures above.

Uses

Two of these electronic voltmeters have been in daily use for two years in the analytical department of the Shell Development Company. The meter is an excellent substitute for a potentiometer in any system with a resistance less than 5000 megohms; it will function as a potentiometer with all the advantages of a continuous reading meter. It has been used satisfactorily to measure the e. m. f. between the electrodes in electrometric determinations involving the use of high-resistance glass, silver, platinum, antimony, and tung-

sten and the corresponding reference electrodes. It is applicable to potentiometric determinations in nonaqueous as well as aqueous solutions and it has no tendency to produce polarization of the electrodes. The meter is well suited for titrating to a definite potential difference, or to a maximum potential change per increment of reagent, and for determining points for plotting potential-volume curves. It is excellently suited for making pH determinations.

Acknowledgments

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Determination of Citral

By Means of the Photoelectric Colorimeter

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CITRAL is an important constituent of lemon flavors. It is present in oil of lemon, which in turn is present in lemon extract.

The federal standard provides that terpeneless extract of lemon shall contain not less than 0.2 per cent by weight of citral (*5*). The Illinois standard specifies the same minimum citral content for imitation and terpeneless lemon extracts and flavors (*2*). For regulatory purposes a quantitative determination of citral is necessary. The official A. O. A. C. or Hiltner method determines citral colorimetrically (*1*). With *m*-phenylenediamine hydrochloride citral forms an intense yellow colored solution, the intensity of which is proportional to the amount of citral present. The amount of citral is determined by comparing this color with that produced by a standard citral solution. In analyzing extracts made with lemon and orange oils, dark-colored solutions sometimes form which mask the resultant yellow color. Parker and Hiltner overcame this by adding some oxalic acid to the reagent, and this improvement is included in the official A. O. A. C. method (*3*).

The method works satisfactorily on unknown solutions that are clear and colorless, but the artificial yellow color of some imitation lemon extracts interferes with observation of the yellow color produced in the course of analysis. Still others are of an emulsion type and contain gums, resins, and starches, in addition to artificial color, which make it impossible to determine citral by comparing the color with that of a standard by the visual colorimeter.

To overcome this difficulty the photoelectric colorimeter was investigated. The one used was the Klett-Summerson (*4*), which is of the double photocell type, the cell current being measured with a potentiometer. The scale is calibrated logarithmically.

When solutions obey Beer's law the relationship between concentration and scale reading is logarithmic. When plotted on ordinary graph paper a logarithmic curve is obtained, while on logarithmic graph paper a straight line is produced. The same result can be obtained by using a logarithmic scale on the instrument; the concentration of solutions obeying Beer's law is directly proportional to the scale reading.

In the preliminary work different strengths of citral solutions were prepared. Aliquot portions were taken and mixed with the *m*-phenylenediamine reagent prepared by the official method. Readings were made on these solutions using various light filters. A blue filter of 420 millimicrons gave the largest difference in scale reading between the lower and higher citral concentrations, and was used satisfactorily in the subsequent work. It was necessary to change the official dilution ratio in order to adapt the A. O. A. C. method to this type of colorimeter.

Reagents

m-Phenylenediamine hydrochloride–oxalic acid solution. Dissolve 1 gram of *m*-phenylenediamine hydrochloride in about 45 cc. of 85 per cent alcohol, and 1 gram of oxalic acid crystals in about the same amount of alcohol. Pour the two solutions into a 100-cc. volumetric flask, add 2.5 grams of fuller's earth, dilute to the mark with 85 per cent alcohol, shake thoroughly, and filter, pouring the fuller's earth into the filter so as to form a filtering medium. Refilter the first 15 to 20 cc. of filtrate through the same filter. This reagent is stable for about 2 days, but after that it is not sufficiently reliable.

Oxalic acid solution. Dissolve 1 gram of oxalic acid crystals in about 90 cc. of 85 per cent alcohol and dilute to the 100-cc. mark with alcohol of the same strength.

Method

Weigh a 10-gram sample and with alcohol transfer to a 100-cc. volumetric flask. Use 95 per cent alcohol for extracts made with lemon oils and 50 to 95 per cent alcohol for terpeneless extracts. Add 10 cc. of *m*-phenylenediamine reagent with an accurate pipet. Complete the volume with alcohol, mix, and pour out a sufficient amount to read in the colorimeter.

Run a sample blank by taking a 10-gram sample in another 100-cc. flask and adding 10 cc. of oxalic acid solution. Pour out a sufficient amount to read in the colorimeter. On clear colorless liquid samples this is unnecessary, as the reading would be zero.

Run a reagent blank by pipetting 10 cc. of *m*-phenylenediamine reagent into a 100-cc. volumetric flask, complete the volume with 50 per cent alcohol, mix, and pour out a sufficient amount to read in the colorimeter.

Use the blue 420 millimicron light filter.

Samples of emulsion type are colloidal when diluted and would give absurd readings. Pour a sufficient quantity from the 100-cc. flask into a centrifuge tube of around 20-cc. capacity, and

TABLE I. ANALYSIS OF STANDARD CITRAL SOLUTIONS

Citral, y, %	Scale Reading, x	Net Scale Reading, $x - a$	Factor, f
0.00	15	...	...
0.06	78	63	0.000952
0.10	120	105	0.000952
0.13	151	136	0.000955
0.16	182	167	0.000957
0.18	204	189	0.000952
0.20	228	213	0.000939
0.22	246	231	0.000952
0.24	266	251	0.000956
0.27	300	285	0.000946
0.30	325	310	0.000968
			Av. 0.000953
0.34	360	345	0.000985
0.40	435	420	0.000953
0.50	530	515	0.000971
0.60	640	625	0.000960

centrifuge until a clear or almost clear solution is obtained. The speed and time will depend on the colloidal stability of the sample. Run the sample blank in the centrifuge at the same time under exactly the same conditions. On cloudy samples a blank should be run, even if without color. Pour off the top liquid for the colorimeter reading.

Calculate from the equation:

$$y = f[x - (a + b)]$$

in which y = per cent citral, f = factor, x = scale reading, a = reagent blank, and b = sample blank.

Note that the sample is diluted tenfold. If the unknown sample was 0.1 or 0.2 per cent solution it becomes 0.01 or 0.02 gram per 100 cc., respectively.

Standard Citral Solutions

Weigh accurately 1 gram of citral and with 95 per cent alcohol transfer to a 100-cc. volumetric flask. Dilute to the mark and mix thoroughly. Pipet 1 or 2 cc. into another 100-cc. volumetric flask, add 10 cc. of *m*-phenylenediamine reagent and dilute to the mark with 95 per cent alcohol, mix, and pour out a sufficient amount to read in the colorimeter. The solution will now contain 0.01 or 0.02 gram per 100 cc., respectively, equivalent to 0.1 or 0.2 per cent of an unknown diluted tenfold. Prepare a series of varying dilutions and with them run a reagent blank as above. Since the standard citral solution from which the set was prepared was colorless, the sample blank is not necessary, as it would be zero. Calculate for the factor, using the same equation: $y = f[x - (a + b)]$.

Once the average factor is determined from the series, it will be unnecessary to repeat the standards.

Experimental

A series of varying dilutions of standard citral solutions was prepared and analyzed on the Klett-Summerson photoelectric colorimeter. The citral used was obtained from the Eastman Kodak Company, Rochester, N. Y., and had a narrow boiling range of 114–115° C. at 25 mm. and a refractive index of 1.489 at 20° C. The average of a series of eight runs is given in Table I. Since a solution containing 0.01 or 0.02 gram of citral per 100 cc. would be equivalent to 0.1 or 0.2 per cent of an unknown diluted tenfold, the standard citral solution may be referred to the corresponding percentages. The 0 per cent citral is the reagent blank, and this reading subtracted from the scale reading for a definite strength of citral gives the net scale reading due to the contact of the *m*-phenylenediamine with the citral. In plotting these net scale readings, a straight line is obtained within the practical range (Figure 1); from the straight-line relationship it follows that this reaction obeys Beer's law. A factor for this relationship can be obtained by dividing the per cent citral by the corresponding net scale reading. The factors for the percentages of citral run (Table I) show a small variation over the range studied, and the average factor, 0.000953, was used in the succeeding calculations. Only one run was made on the 0.34 to 0.60 per cent citral and for that reason was not included in figuring the average factor.

No precautions were taken to assure aldehyde-free alcohol as in the A. O. A. C. method. An increase in reading due to this impurity affects the reagent blank reading by an amount equal to that produced in the citral solution reading, and since the difference of the two readings is used in the calculation, the results would not be affected. The reagent solution deepens in color with age and although still reliable causes variations in the reagent blank readings. This variation does not affect the results, because all readings are greater by the same amount.

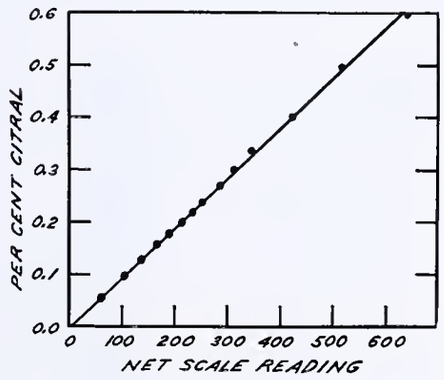

FIGURE 1. RELATION BETWEEN PER CENT CITRAL AND NET SCALE READING

As it was also necessary to run some artificially colored samples, 0.2 per cent citral solutions were prepared and colored with yellow and orange certified dyes. To determine the amount of scale reading due to the dye, the sample was run as before, using oxalic acid solution instead of the *m*-phenylenediamine reagent, since the same amount of oxalic acid was present in the reagent. This is the sample blank. The sum of the scale readings of the reagent blank and the sample blank, subtracted from the scale reading of the sample, will give the net scale reading due to the contact of the *m*-phenylenediamine with the citral. This difference will, when multiplied by the factor 0.000953 previously determined, give the 0.20 per cent citral. Table II shows that the intensity of the yellowness varied greatly, yet the deviation of error was −0.01 to +0.02 per cent and can be attributed to experimental error.

On the market there are so-called lemon creams and emulsions, which vary in color, consistency, and ingredients. Samples were invariably cloudy, and gave erroneous and inconsistent results on reading in the colorimeter. Filtering was not practicable because of the gelatinous nature, volume change due to the alcohol evaporation, and color adsorption by the filter paper. The samples with their blanks were prepared as before, and 15 to 20 cc. were poured into centrifuge tubes with conical bottoms and centrifuged together until

TABLE II. ANALYSIS OF COLORED STANDARD CITRAL SOLUTIONS

Dye Used	Reagent Blank, a	Sample Blank, b	Scale Reading, x	Citral Present %	Citral Found, y %
Colorless solution	10	1	216	0.20	0.20
Yellow 6 (Sunset Yellow)	10	58	268	0.20	0.19
	10	240	480	0.20	0.22
Yellow 2 (Naphthol Yellow S)	10	53	258	0.20	0.19
	10	185	415	0.20	0.21
Yellow 5 (Tartrazine)	10	70	288	0.20	0.20
	10	116	340	0.20	0.20
Yellow 3 (Yellow A B)	10	21	246	0.20	0.21
Yellow 4 (Yellow O B)	10	9	230	0.20	0.20
Orange 1 (Orange I)	10	75	280	0.20	0.19
Orange 2 (Orange SS)	10	31	260	0.20	0.21

Table III. Analysis of Emulsion-Type Colored Standard Citral Solutions

Description	Citral in Original Sample	Citral in Final Sample	Citral Found
	%	%	%
Heavy yellow emulsion	0.042	0.260	0.229
Heavy yellow emulsion	0.080	0.264	0.251
Thin orange emulsion	0.0	0.182	0.179
Thin yellow emulsion	0.0	0.240	0.243

Table IV. Analysis of Commercial Lemon and Orange Flavors

Sample No.	Article	State	Color	Citral %
CD 39	Terpeneless lemon extract	Liquid	Colorless	0.102
MO 180	Lemon extract	Liquid	Colorless	0.227
RS 196	Lemon extract	Liquid	Colorless	0.191
H 358	Terpeneless lemon soda water flavor	Liquid	Colorless	0.116
H 357	Lemon soda water flavor	Liquid	Colorless	0.123
NK 195	Imitation lemon extract	Cloudy liquid	Whitish	0.005
NE 109	Imitation lemon	Liquid	Light yellow	0.008
JJ 389	Imitation lemon	Liquid	Yellow tint	0.006
NE 33	Imitation lemon extract	Liquid	Light yellow	0.000
MC 63	Lemon flavor	Heavy emulsion	Deep yellow	0.042
MO 128	Pure lemon flavor	Heavy emulsion	Yellow	0.080
MO 135	Pure lemon flavor	Thin emulsion	Light yellow	0.000
MO 133	Pure orange flavor	Thin emulsion	Deep orange	0.000
BL 70	Imitation orange flavor	Cloudy and thin emulsion	Light yellow	0.026
HL 7	Pure lemon extract	Liquid	Colorless	0.318
CM 69	Pure lemon extract	Liquid	Colorless	0.281
MC 49	Pure lemon extract	Liquid	Colorless	0.400
HL 28	Pure lemon extract	Liquid	Yellow tint	0.524

clear or almost clear. The top liquors were then poured off for colorimeter readings and found to be low in citral. To the original part of these samples a known amount of citral was added, thoroughly stirred in, and again analyzed. The results are given in Table III. The error varied from −0.031 to +0.003 per cent citral. It is evident that the citral was separated from the gums, starches, etc., by the alcohol and that the yellow color produced by the citral and the reagent was not adsorbed and thrown down with the sediment during the centrifuging.

Application

Commercial samples picked up by inspectors were analyzed for citral (Table IV). These determinations were made to illustrate the application of this method to commercial products of varying compositions.

Summary

The Hiltner official A. O. A. C. method for the determination of citral was modified to make possible the analysis of flavors which contain color, emulsifying agents, or both, with the same degree of accuracy as colorless flavors. Color comparisons were read with the photoelectric colorimeter. Once a factor is determined for the particular photoelectric colorimeter used, standard solutions do not have to be repeated. This has the advantage of being quicker than the visual colorimeter.

These experiments on standard citral solutions show that the solution obeys Beer's law.

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A Convenient Six-Tube Vapor Sorption Apparatus

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THE Forest Products Laboratory has felt the need for a multiple-unit sorption apparatus that could be operated readily over a considerable range of temperatures and used for other vapors than water vapor. The apparatus of Seborg (*4*) in which sorption measurements are made under atmospheric conditions has furnished a great deal of valuable data (*3–6*). The apparatus, however, is not adapted to use over a considerable temperature range and can be used only for water vapor.

The apparatus described in this article was designed at the Forest Products Laboratory to meet these further requirements and to make the attainment of equilibrium as rapid as possible. Diffusion distances were made a minimum (about 40 cm.) and six sorption tubes were arranged so as to be equally accessible to the vapor source. It was considered important to avoid the adding or removing of definite volumes of vapor as many investigators have done because, under these conditions, equilibrium is approached under decreasing or increasing relative vapor pressure conditions which have been shown to affect the results (*7*). Maintaining a definite temperature difference between the vapor source and the samples is the only simple method of holding the equilibrium relative vapor pressure constant as sorption proceeds, that is suitable for various vapors. This method of vapor pressure control was adopted. Quartz spirals (*1*), which have proved their worth in so many different forms of sorption apparatus, were used for the weighing. Although the measurements could be made in this form of apparatus in the presence of air, the rate of attainment of sorption equilibrium was greatly increased by designing the apparatus so that the system could be evacuated and the vacuum maintained.

Apparatus

Figure 1 gives a horizontal plan and a vertical elevation of the apparatus which is mounted in the center of a thermostatically controlled water bath with an inside diameter of 50 cm. (20 inches) and a height of 50 cm. (20 inches). The bath has four vertical side windows, 40 × 10 cm. (16 × 4 inches). None of the control parts for the water bath is shown in the figure. Heating and cooling coils are on the bottom of the tank under the brass turntable gear wheel, *W*, which is supported about 5 cm. (2 inches) above the bottom of the tank. *W*, which supports all the apparatus, is rotated by gear wheel *G*, by turning handle *J*.

A wide-mouthed, unsilvered Dewar flask, *D* (17.5 × 10.5 cm., 7 × 5 inches inside), which serves as the inner bath, is centered on the turntable. This flask is partially filled with a strong aque-

ous glycol solution so as to stand temperatures of −20° C. without freezing and is sealed with a heavy rubber stopper, *Z*, which, for convenience of assembly, is made up of one stopper within another. The bulb, *B*, which serves as the vapor source is centered in the Dewar flask, and connects through a distributing head with six equally spaced tubes (12 mm.) connecting to the sorption tubes, *T*. Each tube is held by ball and socket clamps, *C*, attached to posts, *P*, which are shown only in the horizontal plan to avoid undue complication of the diagram. The six connecting tubes are made up with ground-in joints for convenience in assembly. The sorption tubes, *T*, are also made up with ground-on tops. All these ground joints and stopcocks 1 and 2 are platinum-plated to give better seals with less sticking.

B is equipped with a stirrer, *S*, operated by the electromagnet, *E*. The paddle stirrer, which has been described in detail by the authors (*8*), oscillates slowly back and forth in the liquid, so as to create a new surface continuously without throwing any liquid spray. A mercury manometer, *M*, connected between stopcocks 1 and 2, serves to indicate the vapor pressure of the liquid in *B* at the temperature of the outer bath. *H* is an electrical heating element for the inner bath, *D*. For convenience of varying the heat input, it is connected through a variable voltage transformer to a sensitive relay and an adjustable sealed mercury thermoregulator, *R*.

Y is a cooling coil connected to a refrigeration unit. An expansion valve just above the liquid level in the outer bath prevents undue thermal losses in the line. Since the cooling unit was made entirely of brass tubing, it was necessary to mount a flexible coil above the bath, so that the turntable could be rotated. This was done by bending the brass tubing back and forth in a single plane with about 5-cm. (2-inch) diameter bends and 25-cm. (10-inch) straight sections. The plane of the tubing was then bent around into a circle about 60 cm. (2 feet) in diameter and mounted above the bath with the center line passing vertically through the center of the bath. When the turntable was rotated in one direction the circle merely contracted; on reversing the rotation the circle expanded. The refrigerating unit was set to give a temperature within the inner bath slightly below the desired temperature. The desired temperature was maintained by the heating unit.

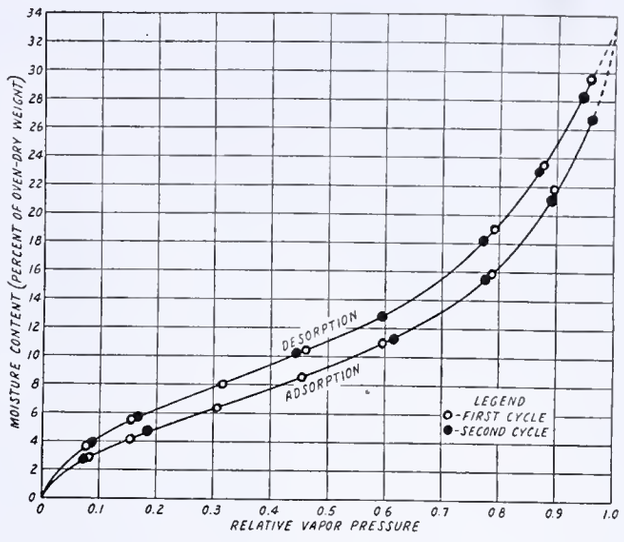

FIGURE 2. SORPTION OF WATER VAPOR BY UNEXTRACTED WHITE SPRUCE AT 20° C.

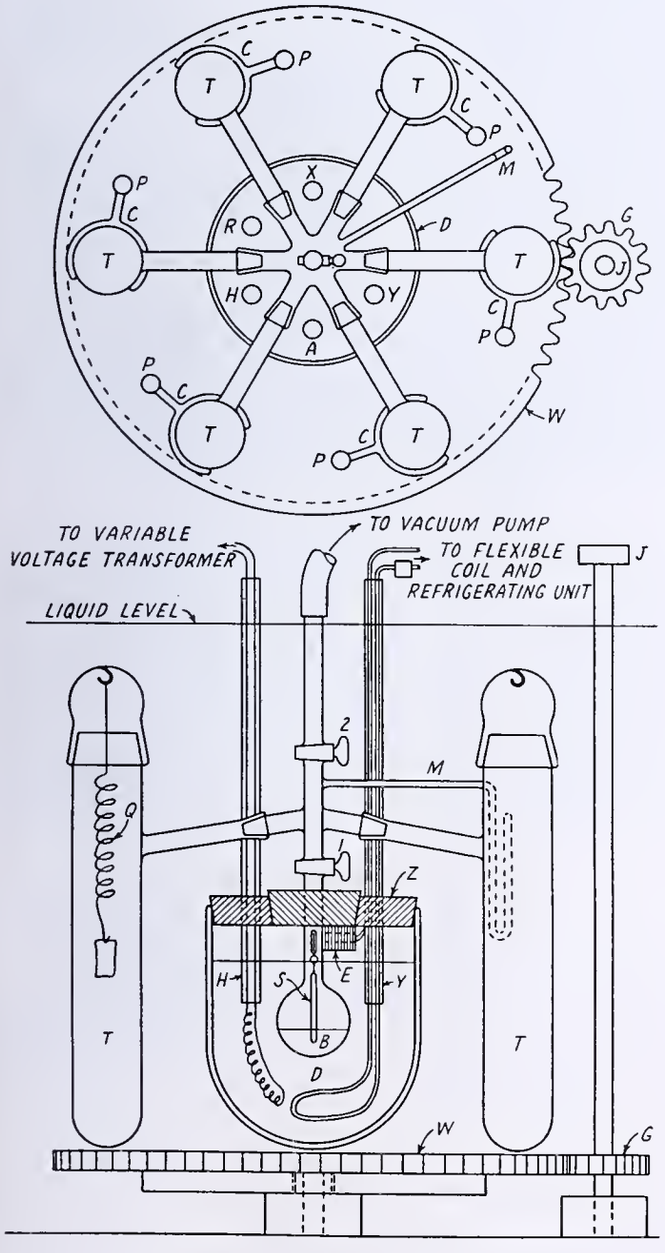

FIGURE 1. SORPTION APPARATUS

The small inner bath was adequately stirred by bubbling air through tube *A*. *X* represents a thermometer well for holding a standardized thermometer which could be read to 0.05° C. When all the controls were properly adjusted, the temperature of the inner bath could be held within an accuracy of at least 0.1° C. at temperatures as low as −8° C. up to the outer bath temperature.

The quartz spirals, *Q*, for following the weight of the samples were 1.2 cm. in diameter and had about 20 turns in a length, under their own weight, of 6 to 8 cm. Under a load of 100 mg. they extended from three to four times their unloaded length. The calibration curves were linear up to loads of at least 100 mg. Moist sample weights never exceeded this value. Tests showed that the extension of the spirals was not affected by changes in relative humidity, as was found by Seborg, Simmonds, and Baird (*5*). This may be due to the fact that a fresh stock of quartz rod was used for drawing out the quartz threads.

The extension of the quartz spirals was measured with a cathetometer permanently mounted in front of one of the outer bath windows. The turntable was rotated so as to bring each tube in succession and also the manometer in line with the cathetometer. Cathetometer readings could be readily checked within an accuracy of 0.005 cm. A 50-mg. load could thus be weighed within an accuracy of 0.05 mg. or 0.1 per cent.

Air-dry samples of the material to be tested (50 to 75 mg.) in a thin sheet form are suspended from the quartz spirals, *Q*. Bulb *B* is one-third filled with distilled water through a fine glass capillary. The apparatus is sealed, stopcock 1 is closed, stopcock 2 is opened, and then the apparatus is evacuated with a high vacuum pump protected with a vapor trap. The water in *B* is frozen, stopcock 1 is carefully opened for a few minutes and then closed. The ice in *B* is melted, stirred, and again frozen, followed by the opening of stopcock 1. After repeating this several times to remove all entrained air, stopcock 1 is kept closed and the outer system is evacuated for 48 hours to bring the samples to constant dry weight. After weighing the samples, *B* is brought to the lowest desired temperature. If this is below the freezing point, the inner stirrer cannot be operated, but at higher temperatures it should be continuously operated. Stopcock 2 is then closed and stopcock 1 opened. The sorption of moisture by the samples from *B*, under the relative vapor pressure determined by the temperatures in the inner and outer baths, was in practically all cases complete in less than 12 hours and in all cases was complete in 24 hours. After determining the equilibrium weights, the temperature in *B* is again raised and the sorption at the newly established higher relative vapor pressure is de-

TABLE I. SORPTION OF WATER VAPOR AT 20° C. BY THREE CELLULOSIC MATERIALS

Relative Vapor Pressure	Adsorption	Desorption	Adsorption-Desorption Ratio
		Spruce	
0.10	3.3	4.2	0.79
0.20	4.9	6.2	0.79
0.30	6.25	7.8	0.80
0.40	7.7	9.5	0.81
0.50	9.25	11.2	0.82
0.60	10.9	13.0	0.84
0.70	12.9	15.5	0.83
0.80	16.3	19.4	0.84
0.90	21.7	25.1	0.86
0.95	25.6	29.0	0.88
			Av. 0.83
		Sulfite Pulp	
0.10	2.7	3.2	0.84
0.20	4.0	4.65	0.86
0.30	5.0	5.75	0.87
0.40	6.05	6.95	0.87
0.50	7.15	8.2	0.87
0.60	8.4	9.6	0.87
0.70	10.1	11.45	0.88
0.80	12.4	14.3	0.87
0.90	16.5	19.1	0.87
0.95	20.9	23.6	0.88
			Av. 0.87
		Cotton Linters Alpha-Cellulose	
0.10	2.1	2.5	0.84
0.20	3.2	3.8	0.84
0.30	3.9	4.6	0.85
0.40	4.75	5.6	0.85
0.50	5.6	6.6	0.85
0.60	6.7	7.8	0.86
0.70	8.0	9.2	0.87
0.80	9.9	11.4	0.87
0.90	13.0	14.9	0.87
0.95	16.9	18.0	0.88
			Av. 0.86

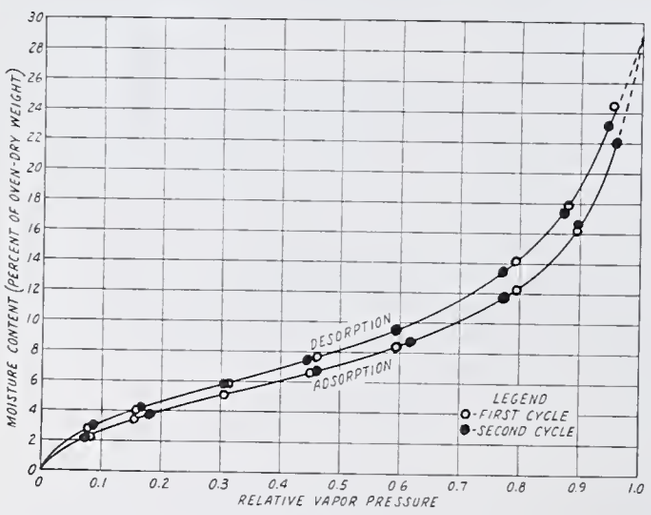

FIGURE 3. SORPTION OF WATER VAPOR BY A COMMERCIAL BLEACHED SULFITE PULP AT 20° C.

termined. The stepwise increases in the inner bath temperature are repeated until the inner bath temperature exceeds the outer bath temperature by a few tenths of a degree. This condition is maintained for 1 to 2 hours to ensure supersaturation of the sample, after which the desorption cycle is followed in a similar manner to the adsorption by decreasing stepwise the inner bath temperature.

Preliminary Sorption Data

Sorption measurements were simultaneously made at 20° C. on two samples of an unextracted white spruce wood two samples of a commercial bleached sulfite pulp, and two samples of a cotton linter alpha-cellulose. The materials were carried through two complete relative vapor pressure cycles. The data are graphically shown in Figures 2, 3, and 4. In all cases the deviations in sorption between the check samples

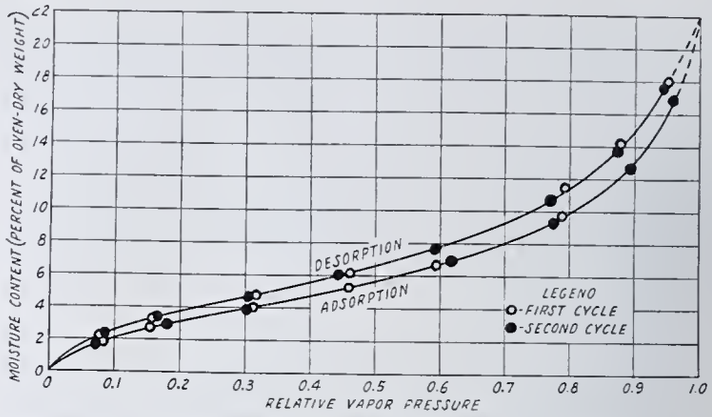

FIGURE 4. SORPTION OF WATER VAPOR BY COTTON LINTERS ALPHA-CELLULOSE AT 20° C.

were less than 0.3 per cent moisture content and averaged about 0.1 per cent; hence they could not be shown on the graphs.

Seborg (*3*) has shown that the ratio of the adsorption to the desorption moisture content is constant over a rather broad range of relative vapor pressures. This is also the case for the data of this investigation, as is shown in Table I. This constancy of the ratio of adsorption to desorption moisture contents holds for relative vapor pressures as low as 0.1 and as high as 0.95. Naturally, it will not hold at the two extremes, as the ratio is unity for these values.

Seborg (*3*) further found the ratio of adsorption to desorption moisture contents to be practically the same for all cellulosic materials and even for lignin. The same constancy is noted for the three cellulosic materials given in Table I. The values given by Seborg (*3*) for cellulosic materials range from 0.82 to 0.89. The three values given in Table I fall within this range.

The chief point of interest here is that Seborg's measurements were made at atmospheric pressure, whereas the measurements given in this paper were made in an evacuated system. This affords further proof (*7*) of the falsity of Patrick's contention (*2*), that hysteresis is due to adsorbed air.

Summary

An improved compact six-tube sorption apparatus is described for sorption measurements under evacuated conditions. Adsorption and desorption measurements at 20° C. are given for unextracted white spruce wood, a commercial bleached sulfite pulp, and a cotton linters alpha-cellulose. The data indicate that the ratio of the adsorption to the desorption moisture contents is constant over a large part of the relative vapor pressure cycle and that the ratios for the different cellulosic materials are similar. This is similar to the findings of Seborg (*3*) for sorption measurements in the presence of air.

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PRESENTED before the Division of Cellulose Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Mo.

An Adjustable Vapor Thermoregulator

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GREEN and Loring (*1*) described a method for constructing an ether vapor thermostat and gave the advantages of using it for room temperature control. They pointed out that the regulator, as described by them, must be constructed at the place where it is to be used.

The modified ether vapor thermoregulator described in this paper has given very satisfactory results for about a year in regulating the temperature in a small room. With a proper relay and heating system, a temperature variation of ±0.02° C. at the thermoregulator can easily be maintained. Its design not only overcomes the objection pointed out by Green and Loring, but also makes it easily adjustable over a temperature range of 20° C. (20° to 40° C.). It can be readily made to cover a much wider temperature range, if desired, by simply lengthening the mercury column.

The vapor thermoregulator, shown in Figure 1, is made of Pyrex glass except for the adjustment mechanism. *A* is a 500-ml. vapor chamber having a bottom tube, *B*, about 65 cm. long and of 12-mm. bore, with a ring seal at *C* to hold the liquid ether in *A*. Tube *D*, about 95 cm. long and of 4-mm. bore, extends through *A* to the bottom of *B*. The adjustment mechanism is connected to *D* a short distance above *A*. *E* is a piece of Jena KPG tubing (of 8.04-mm. bore and 50 mm. in length) with a very uniform inside diameter (±0.001 mm.). (Pyrex tubings with uniform inside diameters are now obtainable.) To the middle of *E* a mercury reservoir, *F*, is carefully attached, so that, except at the seal, *G*, the inside diameter of *E* above and below is not disturbed. Inside *E* is a Fernico alloy plunger, *J*, the diameter of which is only very slightly smaller (about 0.015 mm.) than the inside diameter of *E*, so that it can be moved freely therein by means of a rod connected to a brass bellows, *K* (250 mm. in diameter), without using any lubricant. The metallic bellows is soldered with ordinary solder to one end of a Fernico tube, *L*, the lower end of which is sealed to the Pyrex tubing above *E*. The upper and lower solder joints of the bellows as well as the one to the Fernico tube should be gas-tight. Both the Fernico alloy and the KPG Jena tubing can be sealed directly to Pyrex glass. By turning the thumbscrew *T*, the bottom of the Fernico plunger in *E* can be raised above point *G* or lowered beyond that point. *G* should be about 15 mm. below contact point *H*, so that the mercury always exerts a little pressure against the bottom of the plunger.

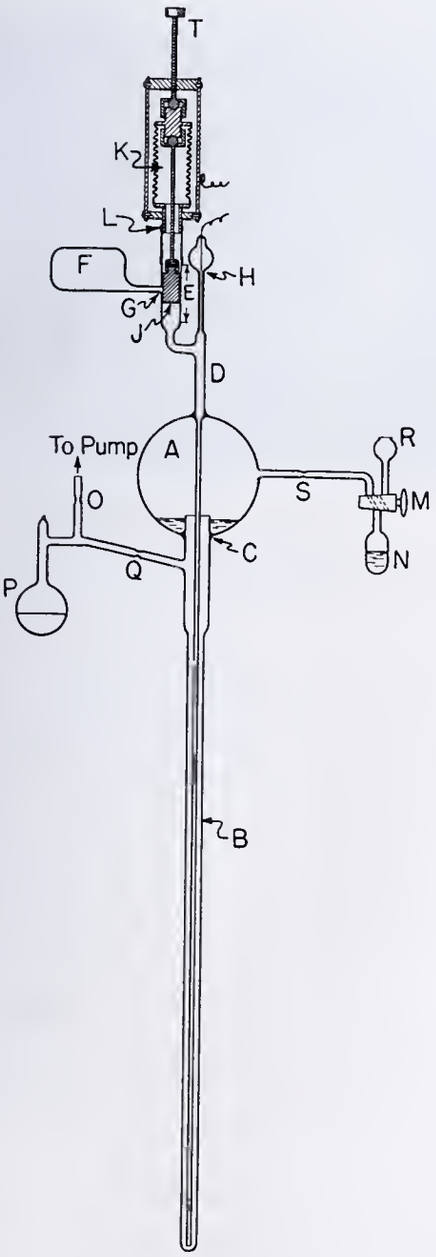

FIGURE 1

After the system has been thoroughly cleaned and dried, mercury and ethyl ether are introduced into the system in the following manner: With stopcock *M* opened to bulb *N*, the system is evacuated with a mercury pump and then sealed off at *O*. Before sealing at *Q*, enough mercury is distilled over from flask *P* to fill tube *B* to a point a little below *Q*. Now cup *R* is filled with dried ethyl ether, about 5 ml. of which are carefully introduced into bulb *N*. Then about 2 ml. of the liquid are distilled into *A* by slightly warming bulb *N*. Some of the ether condenses and remains in tube *B*. The ether in *A* is then cooled with dry ice to reduce the ether vapor pressure in the system before sealing off at *S*.

After removing the dry ice bath from *A*, the instrument is ready for use. Thumbscrew *T* is turned so that the bottom of the Fernico plunger in *E* is a little above *G*. As the thermoregulator begins to warm up to the desired temperature—30° C. for example—the vapor pressure of the ether in the system increases and forces the mercury in *B* to rise in *D*. Since there is enough mercury in this instrument to operate at 20° C., the excess mercury goes into reservoir *F*. When the room temperature is about 0.5° C. below the desired temperature, the plunger is lowered below *G* by turning the thumbscrew to cut off the mercury in the reservoir from the main column. In this respect, the plunger acts as a stopper. It also serves as the final adjustment for the temperature control, which is accomplished by gradually lowering the plunger still further in *E*, so as to force the mercury into the upper part of *D*, until it barely reaches contact point *H*, when the room temperature is exactly at 30° C. Then the distance between *H* and the mercury surface in tube *B* is 63.48 cm., which is the vapor pressure of ethyl ether at this temperature.

The room temperature can easily be raised from 30° to 35° C., by moving the plunger above *G*, to let more mercury into the reservoir *F*, as the temperature in the room is increased. As in the previous operation, the plunger is lowered below *G* again when the room temperature reaches about 0.5° C. below the desired temperature. The final adjustment of the temperature is carried out as before.

To lower the room temperature from 35° to 25° C., the plunger is again raised above point *G*, but this time the mercury in reservoir *F* is allowed to flow back into the system as the room is being cooled, until the room temperature reaches the desired point. The final adjustment is the same as before.

The thermoregulator is flexible. Although contact point *H* is sealed in an evacuated chamber, this thermoregulator (unlike most adjustable thermoregulators of this type) does not require setting in a bath of the desired temperature when changing from one temperature setting to another.

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A Laboratory Water-Bath for Cooking, Mashing, and Fermentation Studies

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THE water baths described in the recent literature (*1*, *2*, *3*) and those commercially available were too small for the authors' requirements in fermentation studies. A bath was desired in which the three stages of cooking, mashing, and fermentation could be conducted with volumes varying from only a few cubic centimeters up to 10 liters. The bath shown in Figures 1 and 2 was designed to satisfy these requirements.

The bath is large enough (300 liters) to accommodate two 12-liter flasks if desired. The over-all dimensions are: 116.2 cm., 46.5 inches (long) × 70 cm., 28 inches (wide) × 66.88 cm., 26.75 inches (high), not including the angle-iron stand. The inside width is 50 cm. (20 inches) and the length is 100 cm. (40 inches) at the widest points. The bath is insulated with at least 5 cm. (2 inches), at the narrowest points, of building insulation wall bats, 37.5 × 57 cm. (15 × 23 inches). The walls are of 0.62-cm. (0.25-inch) Transite with 17 standard aluminum angle 0.3 × 2.5 cm. (0.125 × 1 × 1 inch). The bottom is of white pine.

The inside of the oval bath is tinned copper. The bottom is false and has in the middle, crosswise, a ridge partly visible in Figure 1, *A*, directly under the stirrer. This ridge, 15 cm. (6 inches) higher than the lowest part of the bath, effectively eliminates dead water spots. The depth of the bath at the trough is 60 cm. (24 inches).

The larger glass apparatus is held by two types of baffles which are introduced into slots, *B*, soldered on the sides of the bath, 25 cm. (10 inches) from each end. One type, *C*, holds the tall cylindrical Pyrex jar (45 cm., 18 inches high and 15 cm., 6 inches, in inside diameter), *D*, in which the atmospheric cooking and mashing are carried out. The mash is then transferred for the fermentation to a 12-liter flask which is supported in the bath by a two-pronged baffle, *E* (Figure 2). The baffles are made of polished aluminum sheet, 0.3 cm. (0.125 inch) thick, reinforced at several places with additional aluminum strips. The baffle, *C*, is 23.5 cm. (9 inches) high and 49.67 cm. (19.87 inches) long. The slots attached to the wall are soldered at the lower end.

The top of the bath is covered with copper sheet and refrigerator rubber door gaskets are tacked about each opening upon which rest the aluminum covers, *F*, when the bath is in use. These covers are held in place by the screw fasteners, *G*, serve as another support for the 12-liter flasks, and cover the bath about the tall cylinders when the water is at the boiling point.

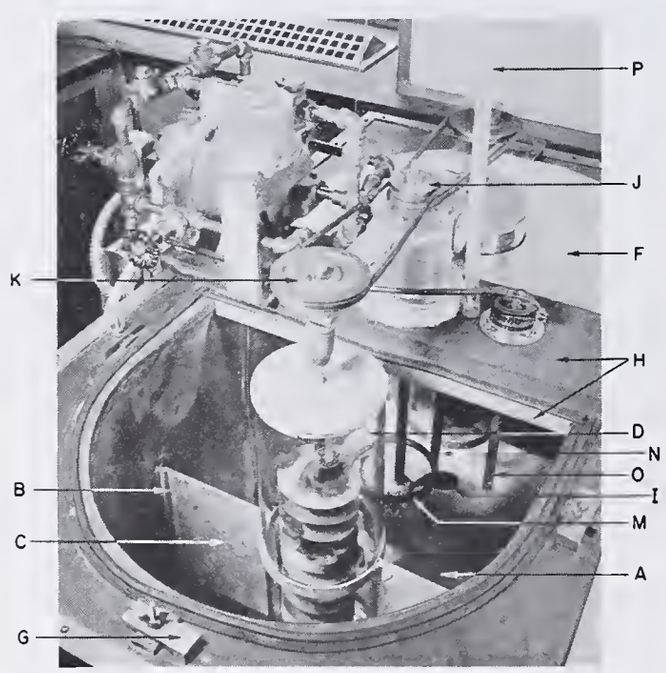

FIGURE 1. WATER BATH FOR FERMENTATION WORK SHOWING COOKING AND MASHING ARRANGEMENT

The bath is emptied by means of two 3.75-cm. (1.5-inch) drains (type K soft copper tubing) and there is also an overflow opening of the same size just underneath the crosspiece, *H*, which is made of a 0.9-cm. (0.375-inch) steel plate, 16.25 cm. (6.5 inches) in width, resting on aluminum angle. The water is stirred by a 20-cm. (8-inch) 3-blade L. H. aluminum propeller, *I*, mounted on a brass shaft. The jar contents, *D*, are stirred by a Monel screw-type stirrer. The brass center pulley, *J*, 5 cm. (2 inches) in diameter, has a speed of 254 r. p. m. The cast-iron end pulleys, *K*, have diameters of 9.4 cm. (3.75 inches) and are mounted on hinges, *L*, to facilitate removal of stirrers and flasks. The stirrers are held by No. 6 Millers Falls straight-shank drill chucks, 0.9-cm. (0.375-inch) cap, along with combination ball bearings and thrust ball bearings.

Figure 1 shows further the steam ring, *M*, directly above the stirrer, the water pipe, *N*, used for filling the bath, and the thermoregulator, *O*, which has a 15-cm. (6-inch) extension (Aminco). The thermometer, *P*, is a special length 101° C. instrument.

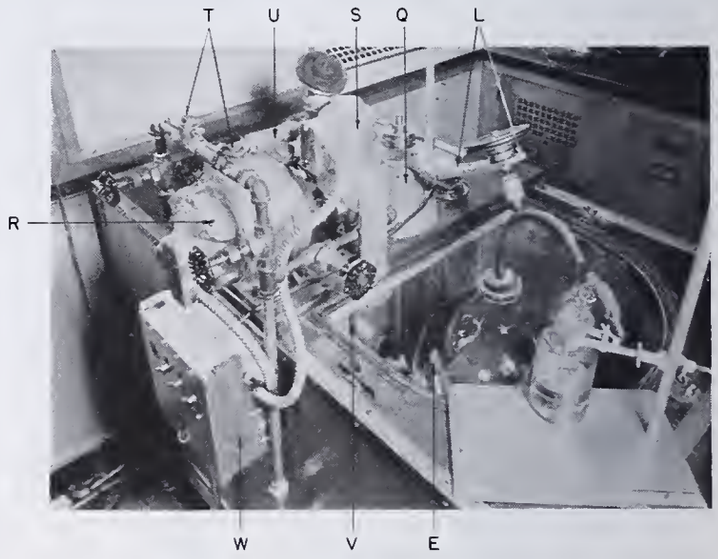

FIGURE 2. WATER BATH FOR FERMENTATION WORK, SHOWING FERMENTATION FLASK AND DETAILS OF ELECTRICAL AND HEATING DEVICES

Figure 2 illustrates the mechanical, heating, and cooling features of the bath. The speed reducer, *Q*, is a Smith No. 2 BV reducer, 7.5 to 1 ratio, with the output shaft extending from the bottom. It is connected with the 0.25-horsepower motor, *R*, through a Boston 3-jaw coupling, 1.25-cm. (0.5-inch) hole, FCN-12. The steam is controlled by a solenoid valve, *S*, which, through a separate pipe system, is also used for cold water control. Both steam and water lines have check valves, *T*, and strainer, *U*. The pipe, *V*, is used for introducing steam under full pressure when it is desired to heat the bath very quickly.

The sheet steel box (20 × 20 × 10 cm., 8 × 8 × 4 inches), *W*, contains all the necessary switches, the double-throw mercury relay (No. 4-376A Aminco), and the transformer (No. 4-460 Aminco Rectran) necessary for operating the thermoregulator. The water line has a connection whereby the water can be passed through a cooling coil for use in hot weather.

To measure the temperature of the mash, a thermometer may be suspended from the edge of the cylinder (Figure 1), or a hole for a thermometer may be bored through the rubber stopper of the 12-liter flask shown in Figure 2.

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A Simplified Microhydrogenation Apparatus

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IN THE course of certain investigations in this laboratory on compounds of plant origin it was desired to carry out quantitative catalytic hydrogenations on small amounts of material. These experiments were designed to determine the number of reducible groupings such as ethylenic double bonds present in the compounds studied, with a view to obtaining structural information concerning them. The various kinds of apparatus previously described for such work have been based on one or the other of two principles: the measurement at constant volume of the decrease in pressure resulting from the consumption of hydrogen, and the volumetric measurement at constant pressure of the hydrogen absorbed.

Apparatus for the manometric procedures were modified from the original Warburg (*15*) manometers for metabolism studies. Hyde and Scherp (*5*) and Kuhn and Möller (*9*) designed apparatus of a complicated nature which gave results of ±2 and ±0.5 per cent accuracy, respectively. Tsuda and Sakamoto (*14*) reported a much simpler apparatus based on the same principle. Foresti (*4*) made use of a solenoid-activated stirring mechanism in an apparatus of high sensitivity.

The earliest volumetric apparatus for microhydrogenations was that of Smith (*13*), who used an arrangement with a compensation flask and differential manometer similar to that in the manometric apparatus and obtained with it an accuracy of ±2 per cent. Slotta and Blanke (*12*) and Bretschneider and Burger (*1*) increased the accuracy of the method to ±1 per cent and introduced several refinements in technique, but produced highly complicated pieces of equipment. Jackson and Jones (*7*) devised new methods of introducing the sample and agitating the reaction flask. Prater and Haagen-Smit (*11*) recently described a new apparatus that can be used with or without the compensation flask.

Simplified apparatus which eliminated the differential manometer and compensation flask was first described by Ebel, Brunner, and Mangelli (*2*). Their apparatus, however, was designed for semimicroquantities of material, as were those of Erdös (*3*), Zechmeister and von Cholnoky (*17*), and Mayeda (*10*). Kaufmann and Baltes (*8*) devised an apparatus on this same principle for determination of the degree of unsaturation of fats by catalytic hydrogenation, using 20- to 40-mg. samples. Weygand and Werner (*16*) used an electromagnetic stirring device in their apparatus.

Description and Operation of Apparatus

The apparatus is made throughout of Pyrex glass. The bulb of the reaction flask is 3.5 cm. in diameter and the neck and side arm are 1.1 cm. in diameter. The ground-in plug, *A*, in the side arm is hollow and to its base is sealed a piece of 2-mm. tubing which serves to retain the sample tube, *F*, in the neck of the flask until the solvent and catalyst have been saturated. Through the side of the plug and the ground surface of the side arm are bored matching holes about 0.5 mm. in diameter. At *B* is a ground-glass connection to a capillary of 1-mm. bore which leads to the measuring buret. This buret is made of tubing of uniform bore, 4 mm. in inside diameter, and about 43 cm. long; its capacity is therefore about 5.5 cc. It is calibrated in 0.1-cc. divisions which are sufficiently long to be read accurately to ±0.01 cc. At point *D*, the base of the buret, a side tube of the same diameter as the buret is sealed. This serves both as a manometer for accurate leveling of the buret liquid and as an inlet tube, when connected at *C* to the source of hydrogen. A leveling bulb is connected by rubber tubing to the apparatus at *E*.

The apparatus is clamped firmly to a 0.94-cm. (0.375-inch) board, about 13.8 × 50 cm. (5.5 × 20 inches), at the points shown in Figure 1. The capillary above *B* is bent out from the board a distance sufficient to accommodate the bulb of the reaction flask, and is supported at *B* by being clamped to a block of wood

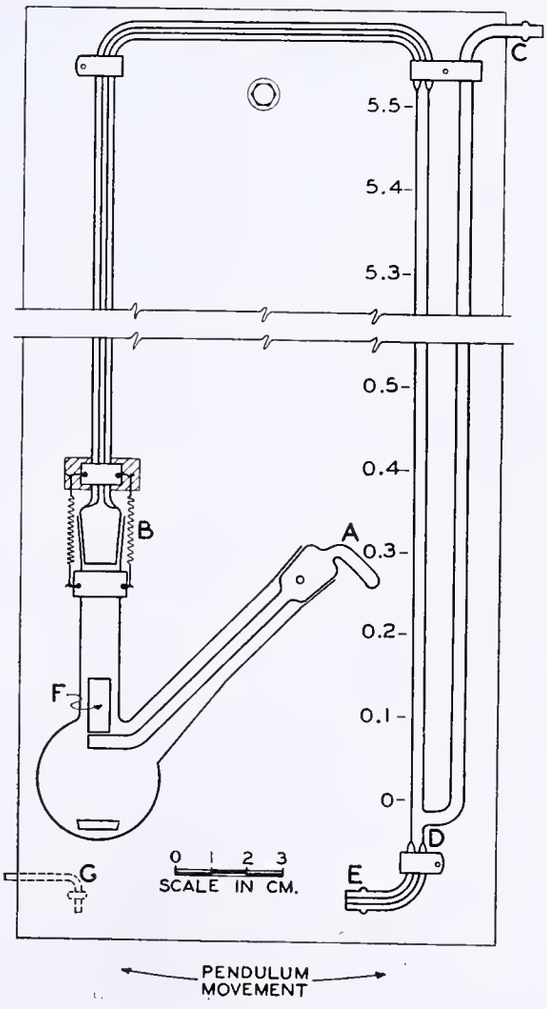

FIGURE 1. APPARATUS

fastened to the board. The board is pivoted and the whole apparatus is agitated by connection to a motor-driven eccentric. A vibration rate of 200 per minute is satisfactory and a variable resistance is connected in the electrical system to control the speed of the motor.

The catalyst, either Raney nickel or Adams and Shriner's platinum oxide, is weighed to the nearest 0.01 mg. in a porcelain microboat and introduced into the reaction flask. Then 5 cc. of solvent are added. The sample, 5 mg. or more, is weighed to the nearest 0.01 mg. in a thin-walled, flat-bottomed glass capsule, *F*, about 8 mm. in outside diameter and 2 cm. long. This capsule is then inserted into the neck of the reaction flask and is held in place by the end of the tube attached to the base of plug *A*, bent as shown in Figure 1. This tube must be so bent that the holes in the plug and side arm meet sufficiently to effect an opening to the apparatus while the capsule is held in position; it must also be possible to turn plug *A* sufficiently to close off the opening through the holes, still retaining the capsule in position. The side arm of the reaction flask must be blown outward above its connection to the flask, as shown in the figure, to allow withdrawal of plug *A*. With *A* adjusted at the open position, the flask is attached to the capillary manifold through the carefully greased connection at *B*, and is held firmly in position by springs. The liquid in the buret, preferably mercury, is lowered below *D* and hydrogen is swept through the apparatus for several minutes, in at *C* and out at *A*. This procedure is a distinct advantage over the method previously used, of alternately evacuating the apparatus and introducing hydrogen, repeating 6 to 10 times.

Before the hydrogen enters the apparatus it is bubbled through a tower containing alkaline stannite solution to remove traces of oxygen and then through a saturator containing the same solvent used in the reaction flask. This procedure reduces to a minimum the time necessary for saturation of the atmosphere in the apparatus. The hydrogen is led through a small-bore tube to the bottom of a side-arm test tube, 17.5 × 2.2 cm. (7 × 0.875 inches), which is filled with glass beads covered by the solvent. It is advisable to insert a Bunsen valve in the inlet tube from the hydrogen cylinder.

When the apparatus has been thoroughly swept out the source of hydrogen is disconnected, plug *A* is closed, still holding capsule *F* in place in the neck of the flask, and the apparatus is let stand until thermal equilibrium is reached. The mercury is then raised until it is exactly at zero in the side tube, *A* is opened momentarily to equalize the pressure in the apparatus and to adjust the mercury at zero in the manometer, and is then closed, still retaining the capsule in the neck of the flask. The catalyst and solvent are saturated with hydrogen, the apparatus being shaken by means of the electrically driven eccentric. Completion of saturation is attained when on continued agitation no further change in the buret reading is observed. This reading is recorded as the initial reading for the hydrogenation of the sample.

Plug *A* is then turned to allow capsule *F* to drop into the solvent, the side opening at *A* being kept closed. Shaking of the apparatus is begun again and the hydrogenation proceeds. Observations of hydrogen consumption may be made at intervals, the shaking being stopped momentarily, and the final reading is made when no further consumption of hydrogen is evident. The temperature at the apparatus is read at each observation, and the barometric pressure is recorded; in hydrogenations requiring several hours the pressure is noted at least at the beginning and end of the experiment. Corrections are applied to the readings as outlined below.

Table I. Vapor Pressures of Common Solvents (*6*)

Temperature	Vapor Pressure			
	CH_3OH	C_2H_5OH	H_2O	CH_3COOH
° *C.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
15	71.0	32.2	12.8	8.5
20	93.3	43.9	17.5	11.7
25	121.6	59.0	23.8	15.0
30	156.9	78.8	31.8	20.6

Calculations

The actual consumption of hydrogen is calculated to standard conditions from the buret readings by the usual procedure, it being necessary to subtract from the barometric pressure the vapor pressure of the solvent at the observed temperature. For convenience the vapor pressures of several common solvents have been taken from the International Critical Tables (*6*) and are presented in Table I. From them curves can be drawn for interpolation at intermediate temperatures.

Corrections to be applied to the readings for changes in temperature or barometric pressure are dependent upon the free volume of the apparatus. This is determined by any convenient method. Temperature changes produce variations in both partial pressure of solvent and volume of the gaseous phase. Corrections for both these factors as well as for changes of temperature may be determined in the following way:

The volume of hydrogen in the apparatus at any time is

$$V_{H_2} = [V_{total} - V_{solvent} - V_{(boat\ +\ capsule)} - V_{buret}] \times \frac{273}{T} \times \frac{P - vp}{760}$$

where P = barometric pressure and vp = vapor pressure of the solvent at temperature T. This volume, V_{H_2}, is calculated at the time the hydrogenation of the sample is started and again at the end. The difference between the two values is the volume of hydrogen, at standard conditions, used by the sample.

Example. Fumaric acid, hydrogen number, 116. Sample, 5.302 mg. Catalyst, PtO_2, 3.112 mg. Solvent, alcohol, 5 cc. Total volume, 35.3 cc. Volume of boat and capsule, 0.4 cc.

Hydrogenation of catalyst: Initial readings, $V_{buret} = 0$; $P = 741.0$; $t = 28.0°$. Final readings, $V_{buret} = 0.69$; $P = 741.3$; $t = 28.5°$.

Hydrogenation of sample: Initial readings, $V_{buret} = 0.69$; $P = 741.3$; $t = 28.5°$. Final readings, $V_{buret} = 2.06$; $P = 741.3$; $t = 28.3°$.

Vapor pressure of alcohol, 70.2 mm. at 28°, 71.5 mm. at 28.3°, 72.5 mm. at 28.5°

$$\text{Initial } V_{H_2} = (35.3 - 5.0 - 0.4) \times \frac{273}{301} \times \frac{741 - 70.2}{760} = 23.94 \text{ cc.}$$

After hydrogenation of catalyst

$$V_{H_2} = (35.3 - 5.0 - 0.4 - 0.69) \times \frac{273}{301.5} \times \frac{741.3 - 72.5}{760} = 23.27 \text{ cc.}$$

$$\text{Observed } V_{H_2} \text{ used by catalyst} = 23.94 - 23.27 = 0.67 \text{ cc.}$$

$$\text{Calculated } V_{H_2} \text{ used by catalyst} = \frac{3.112}{227} \times 2 \times 22.4 = 0.61 \text{ cc.}$$

After hydrogenation of sample

$$V_{H_2} = (35.3 - 5.0 - 0.4 - 2.06) \times \frac{273}{301.3} \times \frac{741.3 - 71.5}{760} = 22.23 \text{ cc.}$$

$$V_{H_2} \text{ used by sample} = 23.27 - 22.23 = 1.04 \text{ cc.}$$

$$\text{Hydrogen number} = \frac{5.302}{1.04} \times 22.4 = 114$$

It is, of course, unnecessary to calculate the volume of hydrogen used by the catalyst. The calculation is put in here to show the close agreement between the observed volume and the volume calculated from the weight of platinum oxide employed. This agreement makes it obvious that catalyst and sample can be hydrogenated simultaneously, correcting the total hydrogen uptake by subtracting the calculated volume of hydrogen used by the catalyst. While the accuracy to be expected by this method is only ±6 per cent, the vigor of the catalyst is considerably greater and the time required for the hydrogenation shorter. Where extreme precision is not necessary the method has some advantages. When using the platinum on zirconium oxide and palladium on zirconium oxide catalysts prepared by the American Platinum Works, the volume of hydrogen absorbed is so small that it can be neglected except where highest precision is required.

Summary

A simple apparatus for quantitative catalytic microhydrogenation is described, and details of the necessary corrections for temperature and barometric pressure changes are given. An accuracy of ±2 per cent, sufficient for determining the number of hydrogenated groupings in organic compounds, is attainable.

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Detection of Carbon Dioxide and Sulfur Dioxide from Mixtures of Carbonates and Sulfites

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IF A GAS containing small quantities of sulfur dioxide and carbon dioxide is passed through a few drops of an acidic solution of ferric ferricyanide, the sulfur dioxide will be oxidized to the nonvolatile bisulfate ion, the brown ferric ferricyanide will be reduced to Turnbull's blue, and the carbon dioxide will pass through the liquid and can be detected with barium hydroxide solution without interference. The simple apparatus (Figure 1) has been developed to carry out the test on a semimicro scale.

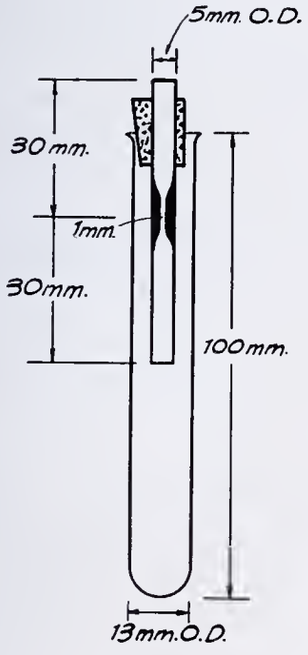

FIGURE 1

Place a drop of a saturated solution of barium hydroxide in the top of the tube and work the drop down into the constricted part. Then put in the lower end of the tube enough of a freshly prepared ferric ferricyanide solution, made by mixing one drop of 0.17 *M* potassium ferricyanide, one drop of 0.3 *M* ferric nitrate, and one drop of 5 *N* hydrochloric acid, to form a thin layer of solution. One or two strands of glass wool may be inserted in the tube to aid in retaining the solution, although they are not necessary.

Carefully remove any liquid on the outside of the tube with filter paper. Put the solid or liquid neutral sample containing about 1 mg. of sulfite and carbonate ion in the test tube, add 4 drops of 5 *N* acetic acid, assemble the apparatus, and place it in a water bath maintained at 70° to 80° for 2 minutes.

If as little as 0.005 mg. of sulfite ion is present the brown solution of ferric ferricyanide will turn blue within a minute. A white precipitate will form in the lower meniscus of the drop of barium hydroxide if as little as 0.025 mg. of the carbonate is present. In the absence of a carbonate, no precipitate forms in the barium hydroxide when as much as 3 mg. of sulfite ion is present. The tube must be cleaned scrupulously after each determination, for even a thin film of ferrous ferricyanide hastens the reduction of the ferric ferricyanide, although the mechanism is not clear.

The solution of ferric ferricyanide should be freshly prepared, for it becomes green after standing 15 to 20 minutes and will no longer oxidize the sulfur dioxide. A similarly prepared solution of ferric ferricyanide was used by Noyes (*2*) to detect the presence of reducing anions in suitably prepared solutions. Noyes states that the blue color is due chiefly to the formation of ferrous ferricyanide. He accounts for the formation of this substance instead of ferric ferrocyanide, which would be expected from the oxidation-reduction potentials, by the slowness of the reduction of the ferricyanide ion by sulfur dioxide. This conclusion is in agreement with the work of Eibner and Gerstacker (*1*), who found that the ferricyanide ion is not reduced by sulfur dioxide even though the solution is boiled. Nitrites, thiosulfates, and sulfides when treated with acids form gases which are reducing agents and will interfere with the test for a sulfite. However, strontium acetate (*3*) may be used to precipitate the sulfite and carbonate ions without precipitating the interfering ions.

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Detection of Gallium

By a Fluorescence Reaction with 8-Hydroxyquinoline

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MINUTE amounts of gallium can be detected by adding 8-hydroxyquinoline to a solution having a pH of 2.6 to 3 and shaking with chloroform. In the presence of gallium, the chloroform layer shows a yellowish fluorescence in ultraviolet light which is due to the extracted gallium hydroxyquinolate. Indium reacts slightly at pH 3 but not at pH 2.6. Other metals do not give an appreciable fluorescence when present in low or moderate concentrations, but a few metals prevent the reaction of gallium.

Procedure

In the absence of ferric iron, cupric copper, vanadate, and molybdate the test may be carried out as follows:

The pH of the solution to be tested is adjusted to 2.5 to 2.6 in any convenient way if indium is present, or to 3.0 if indium is absent (less than 0.05 mg.). The reaction is slightly more sensitive at pH 3.0 than at 2.6. Adjustment of the pH to 3 can be made very simply by acidifying 5 to 10 ml. of the test solution with a drop or two of hydrochloric acid, adding a drop of methyl orange and then a dilute (1 *M*) solution of sodium acetate until the indicator shows the first deviation from its full acid color. One-fourth milliliter of 0.1 per cent 8-hydroxyquinoline solution (obtained by warming 0.10 gram of 8-hydroxyquinoline with 1 ml. of 4 *M* acetic acid until dissolved and then diluting to 100 ml. with water) is added, and the solution is mixed and shaken vigorously with 1 ml. of analytical reagent chloroform in a 1.8 × 15 cm. glass-stoppered flat-bottomed tube. The chloroform is allowed to settle and the layer is viewed transversely while the tube is held vertically over a source of ultraviolet radiation in a dark room. (In the present work a Westinghouse type G-5 ultraviolet lamp was used.) A yellow fluorescence with a tinge of green indicates gallium. When minimal amounts (less than 0.5 microgram) of gallium are to be tested for, the test solution should be compared against a blank, because the chloroform itself fluoresces slightly.

Iron if present in the ferric state must be reduced to the ferrous condition before the gallium test can be applied. This may be done as follows:

One-half gram of hydroxylamine hydrochloride is dissolved in 5 ml. of the slightly acidified solution which may contain as much as 30 to 40 mg. of iron. Sodium acetate solution is added until a brown color appears, and the solution is allowed to stand for 10 minutes (it should become practically colorless in a few minutes). A drop of methyl orange is added, the pH is adjusted to 3.0 (or to 2.6 if appreciable amounts of indium may be present), 0.25 ml. of 0.1 per cent 8-hydroxyquinoline solution is added, and the mixture is shaken with 1 ml. of chloroform. The reduction of iron may not be complete and the chloroform may show a slight brownish color in daylight due to a trace of ferric hydroxyquinolate. So long as the chloroform is only slightly darkened, no harm is done. Prolonged shaking of the solution should be avoided because this leads to reoxidation of the iron.

Vanadium as vanadate can be reduced by hydroxylamine hydrochloride in much the same way as iron. The slightly acidified test solution containing dissolved hydroxylamine hydrochloride is treated with sodium acetate until a greenish color appears, and the solution is allowed to stand until it has become practically colorless. A drop of methyl orange is added, the pH is adjusted to 2.6 to 3, and the solution is then tested as described above.

When copper is present it is precipitated as cuprous thiocyanate by adding potassium thiocyanate and sodium sulfite to the slightly acid solution. Gallium may then be tested for in the filtrate after adjustment of the acidity.

Molybdenum as molybdate can be precipitated with a slight excess of lead nitrate in acetic acid solution, and the filtrate tested in the usual way.

Sensitivity

At pH 3.0 under the conditions described above, 0.1 microgram of gallium in 5 or 10 ml. of solution gives a faint fluorescence that is distinctly stronger than that given by the blank. This corresponds to a concentration of 1 to 10^8. Doubtless gallium can be detected in concentrations less than 10^{-8}, provided the absolute amount is at least 0.1 microgram. The sensitivity at pH 4 is but slightly greater than at pH 3.0. At pH 2.6, in a biphthalate buffer, 0.2 microgram of gallium can be detected with certainty when a blank is used for comparison. The ratio of intensity of fluorescence at pH 2.6 and pH 3.0 is approximately 0.6.

Effect of Other Elements

The following metals give no fluorescence with 8-hydroxyquinoline at pH 3.0 under the conditions already described (25 to 50 mg. of metal in 5 ml. of solution unless otherwise indicated): sodium, potassium, rubidium, copper (Cu^{II}, 10 mg.), silver (nitric acid solution), gold (0.5 mg.), calcium, strontium, barium, magnesium, zinc, cadmium, mercury (Hg^{I} and Hg^{II}, 10 mg.), yttrium (3 mg.), lanthanum (2 mg.), aluminum, thallium (Tl^{I}, nitric acid solution), titanium (Ti^{IV}, 1 mg.), zirconium (1 mg.), cerium (Ce^{III}, 10 mg.), thorium (2 mg.), germanium (0.3 mg.), tin (Sn^{II} and Sn^{IV}), lead, vanadium (V^{V}, 5 mg.), columbium (1 mg. as columbate), tantalum (5 mg. K_2TaF_7), arsenic (As^{V}), antimony (Sb^{III}, 10 mg.), bismuth (10 mg.), chromium (Cr^{III}), molybdenum (Mo^{VI}, 5 mg.), tungsten (W^{VI}, 5 mg.), uranium (U^{VI}), tellurium (0.1 mg.), manganese, iron (Fe^{III}), cobalt (5 mg.), nickel (5 mg.), and platinum (0.2 mg.).

In the case of cupric copper, vanadate, molybdate, and ferric iron, the corresponding hydroxyquinolates are extracted by the chloroform, as can be seen from the color, but the chloroform solution does not fluoresce. Titanium in small amounts gives a precipitate insoluble in chloroform. Lithium appears to give a very faint fluorescence with 8-hydroxyquinoline. One hundred milligrams of lithium (0.75 gram of lithium sulfate) in 5 ml. show about as much fluorescence as 0.1 microgram of gallium. The hue of the lithium fluorescence is more greenish than that of gallium. Beryllium also seems to show a trace of fluorescence. Ten milligrams of beryllium (200 mg. of recrystallized beryllium sulfate tetrahydrate) in 5 ml. at pH 3.0 show a fluorescence (yellow-green) corresponding to 0.1 to 0.2 microgram of gallium. Scandium may give rise to a very faint fluorescence at pH 3.0. Two milligrams of scandium produce approximately as much fluorescence as 0.05 microgram of gallium (fluorescence stronger than the blank but not as strong as 0.1 microgram of gallium).

At pH 3.0, 1 mg. of indium shows about as much fluorescence as 2 micrograms of gallium. The fluorescence of indium is more yellow than that of gallium, the trace of green present in the gallium fluorescence not being evident. The extractability of indium hydroxyquinolate increases rapidly as the pH of the solution is increased above 3. At pH 2.6 (biphthalate buffer) small amounts of indium show no fluorescence. Thus 1 mg. of indium in 5 ml. of solution containing 0.25 ml. of 0.1 per cent of 8-hydroxyquinoline shows a doubt-

Table I. Fluorometric Determination of Gallium

Addition	Gallium Present	Gallium Found
Mg.	*Microgram*	*Microgram*
30 Al	0.0	0.0
30 Al	0.1	0.1
30 Al	0.5	0.55
30 Al	1.0	1.0
50 Al	0.5	0.4
10 Fe^{III}	1.0	0.9

ful fluorescence after shaking with 1 ml. of chloroform, a blank being used for comparison; the fluorescence is less than that of 0.1 microgram of gallium.

The detection of 0.5 microgram of gallium offers no difficulty in the presence of the following metals (25 to 50 mg. present except as indicated): alkalies, alkaline earths, silver, beryllium (10 mg.), magnesium, zinc, cadmium, mercury (Hg^{I} and Hg^{II}, 10 mg.), scandium (2 mg.), yttrium (3 mg.), lanthanum (2 mg.), aluminum, indium (1 mg.), thallium (Tl^{I}), zirconium (1 mg.), cerium (Ce^{III}, 10 mg.), thorium (2 mg.), lead, arsenic (As^{V}), chromium (Cr^{III}), tungsten (W^{VI}, 5 mg.), uranium, manganese, cobalt (5 mg.), nickel (5 mg.), platinum (0.2 mg.), and palladium (0.2 mg.). It is believed that less than 0.5 microgram of gallium can be detected in the presence of most of these metals even when they are present in amounts greater than those specified. In the case of tin, antimony, and bismuth, which yield heavy precipitates by hydrolysis, 1 to 2 micrograms of gallium are required for a definite reaction when 10 mg. of one of these metals are present. The precipitate in the chloroform makes it difficult to recognize a fluorescence, and these elements are best separated before applying the test. Titanium, columbium, tantalum, and tellurium also should be separated unless present in very small amounts.

The metals interfering seriously with the detection of gallium are those already mentioned as forming hydroxyquinolates soluble in chloroform under the conditions of the test—namely, ferric iron, cupric copper, vanadate, and molybdate. By reduction or precipitation of these metals as described above, it is possible to detect 0.5 microgram of gallium in the presence of 30 mg. of iron, 5 mg. or more of vanadium, and 10 mg. of copper. One microgram of gallium can be detected in the presence of 5 mg. of molybdenum.

Fluoride reduces the sensitivity of the gallium reaction enormously. In 5 ml. of solution of pH 3.0 containing 10 mg. of sodium fluoride, 2 micrograms of gallium are barely detectable. However, if aluminum is present in sufficient amount, the sensitivity is not reduced. Citrate also inhibits the reaction. Phosphate reduces the sensitivity only slightly. One-half microgram of gallium in 5-ml. solution of pH 3 containing 100 mg. of ammonium monohydrogen phosphate still gives a fluorescence, but a weaker one than in the absence of phosphate. With 10 mg. of ammonium monohydrogen phosphate in 5 ml., 1.0 microgram of gallium gives approximately the same intensity of fluorescence as 0.9 microgram in the absence of phosphate.

Quantitative Application

Preliminary experiments indicate that the reaction described may be applied to the determination of small quantities of gallium in the presence of relatively large amounts of such elements as aluminum and iron. The results given in Table I were obtained by fluorometric titration.

A dilute standard solution of gallium was added to a comparison solution having the same volume and pH as the unknown solution, and containing the same amount of 8-hydroxyquinoline and chloroform, with shaking after each addition until the chloroform layers in both solutions showed the same intensity of fluorescence. The solutions were adjusted to pH 3.0 and ferric iron was reduced with hydroxylamine hydrochloride. The comparison solution did not contain aluminum or iron.

There appears to be but little diminution in the fluorescence intensity of gallium in the presence of moderate amounts of aluminum or iron. Zinc, however, reduces the intensity of the gallium fluorescence markedly. Thus, 1.0 microgram of gallium in the presence of 20 mg. of zinc (5-ml. volume, pH 3.0) gave approximately as much fluorescence as 0.5 microgram of gallium in a zinc-free solution. Therefore, if gallium is to be determined in the presence of zinc without making a separation, approximately an equal amount of zinc must be present in the comparison solution.

Micromethod of Chromatographic Analysis

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THE chromatographic method of analysis originated by Tswett (*9*) and revived by Kuhn and associates (*5*, *6*) has proved a valuable physical method for the isolation and purification of substances from mixtures.

Considerable difficulty is sometimes encountered and large quantities of material are required in the selection of suitable solvents, adsorbents, and elutriants for the substance to be investigated. A microchromatographic method that requires only a few drops of material has been developed, and has been used in this laboratory for the past two years for rapid preliminary survey before filtering solutions through columns in preparation for spectroscopic examinations.

A number of adsorbents are placed in the cups of a "spot plate" or cupped porcelain dish such as water colorists use. A very small

Figure 1. Top View of Petri Dish, Showing Zone Formation in Adsorbent Material

quantity (0.25 teaspoon) of the adsorbent in the powdered form is placed in the cup and moistened with various solvents; 2 or 3 drops of a prepared creamy mixture of adsorbent and solvent may be used. A drop or two of the solution to be investigated is then placed at the rim of the cup and allowed to flow into the adsorbent; the combination that is most suitable for the particular substance to be studied is determined.

Then a glass Petri dish about one quarter full of the chosen adsorbent is gently shaken in a tilted position, so that the adsorbent settles in the form of a wedge that is very thin at the upper edge and a few millimeters thick at the lower edge. The solution that is to be analyzed is dropped from a 1-ml. pipet into the center of the Petri dish, which is held in a tilted position (Figure 1) so that the solution may flow gently towards the thin edge of the wedge and then filter slowly downwards through the adsorbent. The solvent is added drop by drop to form broad zones of separated material. Experience will determine in each case whether it is better to allow the solution to flow into the adsorbent in the dry state or when moistened slightly with the solvent.

If material that contains fluorescing substances is used, these spread out into broad semicircular fluorescing zones in the light of a Hanovia analytic quartz lamp. This method is very useful in the analysis of biologic materials when only very small quantities are available, and has been found more satisfactory than other micromethods (*1–4, 7, 8, 10*) in the analysis of certain biologic substances.

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Modified Electrometric Determination of Metallic Silver

By a Dead-Stop End-Point Procedure

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NO CHEMICAL method is known for determination of metallic silver in suspensions, such as one would have in exposed photographic emulsions, if silver is present to the order of 1 to 10 $\times$ 10^{-9} mole per liter. A volumetric method can now be reported for estimating such small amounts of silver which makes use of the polarized-electrode method of Foulk and Bawden (*1*). This method measures metallic silver only and is not affected by the presence of silver ions. The reactions involved in the determination are:

$$2Ag^{\circ} + I_2 \rightarrow 2AgI \qquad (1)$$

$$I_2 + Na_3AsO_3 + H_2O \rightarrow Na_3AsO_4 + 2HI \qquad (2)$$

The silver iodide formed is dissolved by the excess potassium iodide and any hydrogen iodide is neutralized by the sodium bicarbonate. At such low concentrations of silver, special precautions to reduce errors are necessary. A method of following the titration was developed which allows greater precision to the end point. This paper is an account of these factors.

Titration of iodine using the dead-stop end point may be divided into four categories—addition of an iodine solution to a reducing agent in an acid or in an alkaline medium; and addition of a reducing agent to an iodine solution which is acid or alkaline. Wernimont and Hopkinson (*2*) report that titration of iodine with sodium thiosulfate in an acid medium is not so reproducible as when the reverse titration is carried out. The authors studied the titration of iodine and arsenite solutions from both directions and found both end points repeatable.

Experimental Procedure

All chemicals used were of reagent quality. The alkalinity of solutions to be titrated was adjusted to pH 8.3 with 0.02 *N* sodium bicarbonate. Solutions were made with freshly distilled conductivity water and stored in Pyrex bottles. The iodine

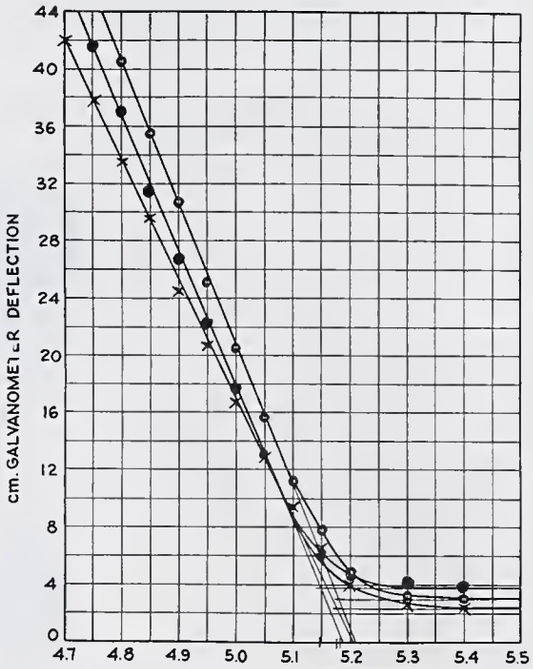

FIGURE 1. TITRATION OF IODINE WITH ARSENITE
30 per cent KI, 0.02 *M* $NaHCO_3$
E. m. f., 100 millivolts

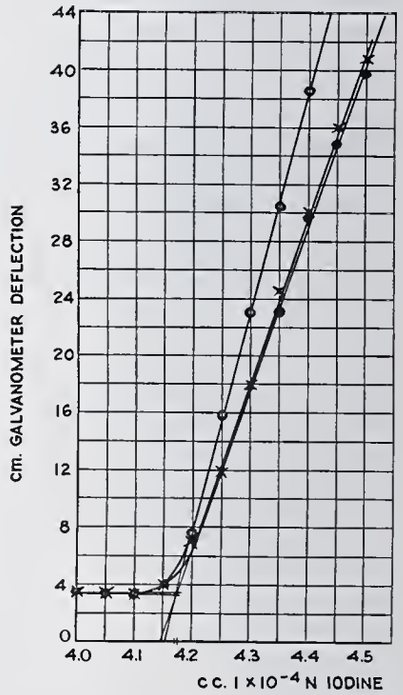

FIGURE 2. TITRATION OF ARSENITE WITH IODINE
30 per cent KI, 0.02 *M* $NaHCO_3$
E. m. f., 100 millivolts

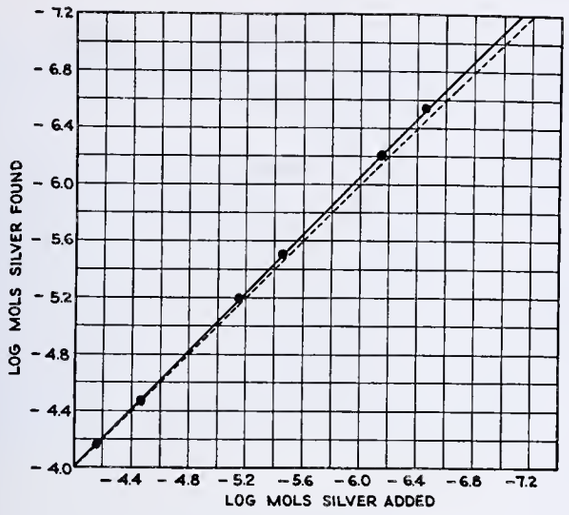

FIGURE 3. DETERMINATION OF SILVER

0.02 *M* $NaHCO_3$, 30 per cent KI
Dashed line indicates no shortage in silver found

solutions contained 40 grams of potassium iodide per liter to increase their stability.

The apparatus was similar to that of Wernimont and Hopkinson (*2*) with the addition of a variable shunt across the galvanometer in order to adjust its sensitivity suitably over a wide range of concentrations. The galvanometer used was the Leeds & Northrup type P, of 1171 ohms' resistance and sensitivity of 8×10^{-10} amperes per mm. A precision dial was attached to a type 371 (General Radio Company) rheostat-potentiometer of 900 ohms' resistance for adjusting the voltage supplied by three dry cells connected in parallel to the electrodes. By calibrating the dial, voltages across the electrodes were ascertained.

It was found necessary to maintain an atmosphere of nitrogen over the solution in the titration vessel and to bubble nitrogen through silver bromide sols during exposure (when silver was obtained from this source). The nitrogen was purified by passing it through a train of chromous sulfate and acid silver nitrate containing suspended silver bromide. Oxygen was removed by the chromous sulfate, sulfur by silver nitrate, and the silver bromide effected the removal of some agent which was able to reduce silver bromide even in the absence of light. The treatment of the nitrogen must be very efficient, since traces of oxygen cause oxidation of potassium iodide and the resulting iodine gives a spurious end point.

The dial is adjusted to the desired voltage and either iodine or arsenite solution is placed in the titration vessel. In the first case, a large deflection of the galvanometer is immediately observed. Addition of small increments of arsenite causes the galvanometer to move towards its rest point (to some extent affected by the voltage across the electrodes) by definite amounts. In the second case, a momentary large deflection of the galvanometer takes place but it quickly returns to a position near zero deflection (not appreciably influenced by voltage). When the end point is reached, small increments of iodine cause the galvanometer to move away from its rest point by definite amounts. A record is made of cubic centimeters of solution added and of galvanometer deflection.

Graphical Treatment of Data

In Figures 1 and 2 curves of such data are shown. Experiments were in triplicate. It is apparent that a linear relationship exists, over a wide range of titration, between amount of titrant added and galvanometer deflection. If this straight-line portion is extrapolated to the base line which represents the minimum galvanometer deflection, the intersection read off on the volume axis of the graph is the end point.

A significant difference is observed in the shape of the toe of the curves in the two sets of data. Whereas, when arsenite is added to iodine (Figure 1) a very broad curvature obtains, the reverse titration (Figure 2) shows a very sharp curvature. This makes it apparent that with the former a sharp end point would be hard to obtain by merely watching the galvanometer come to rest, while the latter shows a permanent deflection of the galvanometer only where the end point is very nearly reached. Even with this graphic method the data show that titration of arsenite with iodine is relatively more reproducible than the reverse procedure. No attempt was made to adjust the iodine solution equivalent to the arsenite. In fact, the end points for such equivalent solutions would not be the same for the reverse titrations. Thus, iodine must first be standardized against arsenite, and in an analysis the titration must be carried out in the direction used for standardizing.

Reliability of Silver Determination

A colloidal silver sol, carefully freed of silver oxide and other possible sources of silver ions, was prepared and its silver content was determined by solution in nitric acid and potentiometric titration of the resulting silver ions with potassium iodide. The stock silver sol was diluted in various amounts with conductivity water carefully freed from oxygen and the silver content of the diluted sols was measured, using the method described in this paper. The data are shown

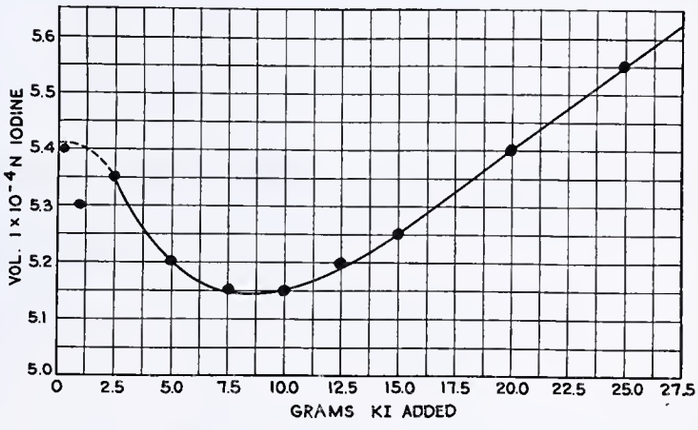

FIGURE 4. EFFECT OF POTASSIUM IODIDE CONCENTRATION ON END POINT

0.02 *M* $NaCO_3$, solutions 1 × 10^{-4} *N*
E. m. f., 100 millivolts

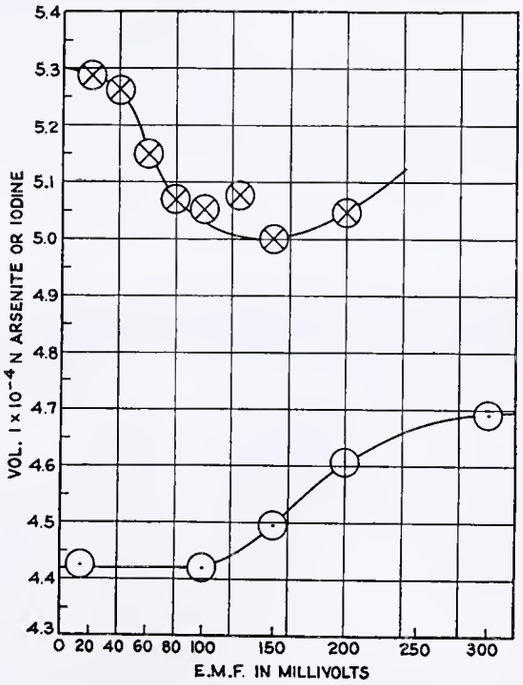

FIGURE 5. EFFECT OF E. M. F. ON END POINT

0.02 *M* $NaHCO_3$, 30 per cent KI
⊙. Iodine titrated with arsenite
⊗. Arsenite titrated with iodine

graphically in Figure 3 and indicate that the new method is reliable over a considerable range of silver concentrations. The slight deviation from the theoretical line may be due to traces of dissolved oxygen in the water used for dilution.

Influence of Potassium Iodide Concentration

In order to determine photolytic silver in silver halide sols free from gelatin it is necessary to dissolve the sol in nearly 30 per cent potassium iodide. This factor was studied from 0 to nearly 50 per cent of iodide. The result is shown in Figure 4. The data at low iodide concentration are somewhat erratic and this appears to be caused by nonuniform sampling of the solid iodide. Use of a stock solution eliminates the difficulty. Above 5 per cent, however, samples are uniform and the solid salt may be used.

Effect of Voltage across the Electrodes

The voltage across the electrodes affects both the end point and the slope of the straight-line portion of the titration curve. The slopes for titration from either direction increase as the voltage increases up to about 100 millivolts, after which they remain relatively constant.

The end point, on the other hand, is different in the two cases, as is shown in Figure 5. Titrations at any potential are reproducible but the potential should be carefully adjusted for the most precise results.

Accuracy of Estimation of Metallic Silver

In the titrations described above, 0.0001 N solutions were used. When 0.00001 N solutions were tried, the electrode action became somewhat sluggish but the end point was definite and reproducible. From Figure 2 it would appear that titrations may be reproduced to ± 0.002 cc. but in actual silver determinations the authors were not able to check better than ± 0.01 cc. of 0.0001 N iodine, which is equivalent to $\pm 1 \times 10^{-9}$ mole of silver. Five micrograms of silver can be determined to ± 1 per cent or 0.5 microgram of silver to ± 10 per cent.

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Communication No. 801 from the Kodak Research Laboratories.

Iodosulfate Microchemical Identification Tests for Cinchona Alkaloids

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THE identification of alkaloids and other amines by microscopical observation of their crystalline precipitates has been developed to a high degree. In the past these tests have been made almost invariably on aqueous solutions, although usually the dry substance is available, either submitted as powder, pill, or capsule in the first place, or isolated by extraction. Sometimes the best identifying crystals are obtainable from strong acids without admixture of water; and often it is easier to hold the acidity, concentration of the precipitating compound, and other factors within the limits giving the best crystals if the reagent embodies these conditions and can be applied directly to the solid alkaloid or its salt. Accordingly reagents for direct application are being developed for various alkaloids.

Among the advantages of microscopic crystal tests an important one is the definite discrimination of isomers or closely related compounds. Not all such tests will accomplish this, for often there is a family resemblance even in the crystal forms. Usually, however, some reagent can be found or devised which will readily and clearly distinguish between a particular pair of isomeric alkaloids. The tests of this article yield entirely different crystals with quinine and quinidine, cinchonidine and cinchonine.

The microscopic tests in present use or recommended for these four alkaloids are made on their aqueous solutions, except for some variations of the herapathite test. The best known may be found in the books by Stephenson (*14*) and Amelink (*1*). A number of the tests they give were due originally to Behrens (*3*) or Grutterink (*8*). These may be supplemented by certain suggestions of the writer: chloromercuric acid with 15 volumes per cent of hydrochloric acid for quinine (*6*), bromoplatinic acid for cinchonine (*6*), dilute (0.2 per cent) picric acid for cinchonine (*7*), and ferrocyanide with phosphoric acid for cinchonine and cinchonidine (*7*). Recent studies, with new recommendations, have been made by Whitmore and Wood (*16*) and Martini (*11*). There does not appear to be as yet any general agreement as to the best test for any one of the four alkaloids.

The crystals of quinine iodosulfate, or herapathite, so notable and well known for their extreme dichroism, provide certain identification of quinine when they can be obtained. Even without a microscope they are characterized by a dark beetle-green metallic luster or iridescence. Numerous variations of this quinine test have been given since its discovery by Herapath (*1, 2, 4, 9, 10, 12, 15*). Mulliken (*13*) and Fuller (*5*) state that cinchonidine in this test yields microscopic needles without metallic luster, and that quinidine also yields an iodosulfate precipitate, which according to Fuller is reddish brown and much more soluble than that of quinine, so that it often takes a considerable time to form. Quinidine may not give the test, depending on the variation used. When quinidine iodosulfate crystals are obtained they do not resemble herapathite in optical properties.

In the writer's experience none of the previous versions of the herapathite test has been very satisfactory, even for quinine alone, on the small scale used for other microcrystal tests for alkaloids. His purpose at first was to perfect this quinine test for microchemical use. It was found that excellent iodosulfate crystals could also be obtained with quinidine, cinchonine, and cinchonidine.

Quinidine, the stereoisomer of quinine, gives canary-yellow, nondichroic crystals. Cinchonine gives plate crystals varying in depth of color but also nondichroic, as observed with polarized light. Of the isomers cinchonine and cin-

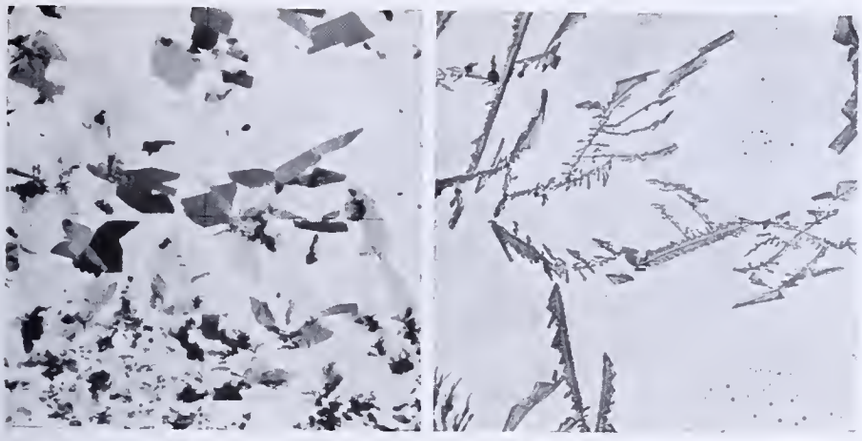

FIGURE 1. QUININE CRYSTALS (HERAPATHITE), WITH POLARIZED LIGHT (*left*); QUINIDINE CRYSTALS (*right*)

FIGURE 2. CINCHONINE CRYSTALS, RECTANGULAR AND PENTAGONAL TYPES (*left*); CINCHONINE CRYSTALS, TRIANGULAR TYPE (*right*)

chonidine, cinchonidine corresponds the more closely to quinine, cinchonine to quinidine. Cinchonidine gives two distinct kinds of crystals, under different conditions, needles and plates, the latter showing dichroism as extreme as that of herapathite. This dichroism is an almost complete absorption of light vibrating in one direction in the crystal, with transparancy to light vibrating at right angles. All the crystals are highly birefringent, and appear bright with crossed nicols.

Method

Put a small amount of the dry, powdered alkaloid or its salt in a little heap on the microscope slide, add a drop of reagent, and apply a cover glass. The tests are sensitive, but the characteristic crystals are formed at a fairly high ratio of alkaloid to reagent, at least for quinine and cinchonidine; hence the amount used is put in a small heap, not scattered over the slide. Crystallization takes place immediately with quinine, quickly (in most cases) with the others. Examine the crystals under a microscope, with a magnification of 50 to 100, making examination by polarized light if possible (using only the polarizer or only the analyzer) in order to observe the dichroism. However, the crystals are characteristic and can be used for identification with ordinary light.

Solutions for Making Reagents

WAGNER'S NO. 1. Iodine, 1 gram; potassium iodide 1 gram; water, 100 cc. Dissolve the iodine and potassium iodide completely in 1 to 2 cc. of the water, add a few excess iodine crystals, and dilute with the rest of the water.

WAGNER'S NO. 2. Iodine, 1 gram; potassium iodide, 1.75 grams; water, 100 cc. Dissolve the iodine and potassium iodide in 2 to 3 cc. of the water, then dilute with the rest of the water.

IODINE IN (2 + 1) ACETIC ACID. Dissolve 0.5 gram of iodine in 50 cc. of glacial acetic acid (this may take several days, with occasional shaking); then dilute with 25 cc. of water. Leave in contact with excess iodine crystals.

Glacial acetic acid, water, and diluted sulfuric acid (1 + 3): one part by volume of concentrated sulfuric acid plus 3 parts by volume of water.

Reagents and Crystals

IODINE REAGENT Q. Wagner's No. 2, 3.0 cc.; glacial acetic acid, 1.5 cc.; sulfuric acid (1 + 3), 1.5 cc.

Quinine Crystals. Plates, mostly of a pale olivaceous green tint by ordinary light, red where they overlap. By polarized light they are variously colored; practically colorless, yellowish, greenish, olive-green, gray-green, pink, red, and black. Rotation of the stage or of the nicol discloses the pleochroism, colorless or greenish to dark red or black. The photomicrograph (Figure 1, left) is by polarized light. The test is sensitive and only a little quinine need be used; but the characteristic crystals are seen at the spot of greatest concentration. Out in the solution, where there is excess of iodine, brown colored crystals may form.

Quinidine Crystals. Canary-yellow plates, not dichroic, of various irregular shapes. Figure 1 (right) shows one type. Crystals vary somewhat in shape with this and the following reagents, but are nearly uniform in color.

This reagent may yield crystals with cinchonine and cinchonidine, but does not give dependable tests for them.

IODINE REAGENT C-1. Wagner's No. 1, 4.4 cc.; iodine in (2 + 1) acetic acid, 3.1 cc.; glacial acetic acid, 2.1 cc.; water, 1.8 cc.; and sulfuric acid (1 + 3), 0.6 cc.

Quinine Crystals. As with the preceding reagent, but usually smaller and more densely matted together.

Quinidine Crystals. As with the preceding reagent, but generally thinner and more branched and irregular in shape.

Cinchonine Crystals. Plates, commonly red-brown in color, varying to red, brown, black, and yellow; scarcely if at all dichroic. They are of several different but definite shapes, the rectangular and pentagonal generally predominating with this reagent (Figure 2, left), sometimes the triangular (Figure 2, right), the latter usually predominating with reagent C-2. Reagent C-1 is the best of the three reagents for cinchonine. The test is sensitive and usually gives numerous crystals in various areas under the cover glass; but occasional difficulty in readily obtaining crystallization has been experienced. Submicroscopic seeding, even just by the reuse of slides, washed and then wiped

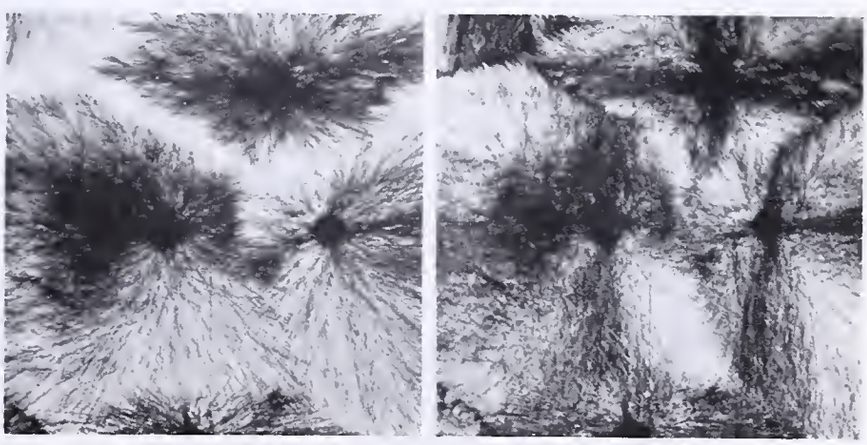

FIGURE 3. CINCHONIDINE NEEDLE CRYSTALS, REAGENT C-1 (*left*); CINCHONIDINE NEEDLE CRYSTALS WITH CROSSED NICOLS, SHOWING POLARIZATION CROSSES (*right*)

FIGURE 4. CINCHONIDINE PLATE CRYSTALS, WITH ORDINARY LIGHT, REAGENT C-2 (*left*); CINCHONINE-CINCHONIDINE MIXED CRYSTALS, REAGENT C-1 (*right*)

dry, on which crystals have been obtained, seems to be enough to obviate this difficulty. Crystals of one of the other alkaloids can be used for this seeding.

Cinchonidine Crystals. Rosettes of light brownish needles, showing with polarized light a dichroism in tint, purplish one way and yellowish at right angles. These form gradually, at or near spots of highest alkaloidal concentration (Figure 3, left). With crossed nicols they show marked polarization crosses, the directions in a rosette of the purplish and brightest yellow colors giving the dark arms of the cross (Figure 3, right). The crosses revolve like the spokes of a wheel when the stage is rotated between crossed nicols.

IODINE REAGENT C-2. Wagner's No. 1, 3.3 cc.; iodine in (2 + 1) acetic acid, 4.4 cc.; water, 0.5 cc.; and sulfuric acid (1 + 3), 3.8 cc.

Quinine Crystals. Similar to the description given above.

Quinidine Crystals. As previously described, but not forming as readily, and tending toward more regular forms of slightly deeper color, sometimes orange-yellow prisms.

Cinchonine Crystals. See description under the preceding reagent, and Figure 4 (right). In a few tests bunches of yellowish-brownish branching threads, and some splinter crystals, also formed.

Cinchonidine Crystals. Dense black rosettes of pointed plates. Individually (as seen at the edges of the rosettes or with the occasional separate plates, which are little pointed ellipses) the crystals are pale gray or yellowish gray in ordinary light. With polarized light the separate crystals vary, and with rotation change from virtually colorless and almost invisible to black and perfectly opaque. The photomicrograph (Figure 4, left) shows better developed crystals than most, and was taken by ordinary light to show the structure of the rosettes.

Mixtures

The tests, like others of their kind, are primarily intended to make identification absolutely certain, not to provide for detection in mixtures. Nevertheless, as herapathite is very insoluble and yet crystallizes readily, quinine, even in small proportion by weight, can be identified in many mixtures without separation, whenever it predominates in the precipitation. In general, the presence of some other alkaloid or amine that precipitates with the reagent will cause interference, and in any considerable amount may ruin the tests for the other cinchona alkaloids. Numerous other alkaloids precipitate and yield crystals, the reagents then acting like iodine–hydriodic acid rather than iodosulfate reagents. Other crystals, so far as known, do not resemble those described.

In the presence of one another the cinchona alkaloids give their characteristic crystals as follows:

Quinine, from a mixture containing up to twice as much quinidine, three times as much cinchonine, four or five times as much cinchonidine. Quinidine, from a mixture with an equal amount of quinine, two and one-half times as much cinchonine, or three times as much cinchonidine. Cinchonine, when constituting 50 per cent or more of mixture with cinchonidine, or 70 per cent with quinine or quinidine. Cinchonidine to give its characteristic crystals must constitute about 65 per cent of a mixture with cinchonine, about 80 per cent with quinine or quinidine. Cinchonidine tends to crystallize with the other alkaloids instead of yielding its own crystals. The herapathite crystals are actually improved by a large admixture of cinchonidine with the quinine. The quinidine crystals become smaller, more grainlike, and a deeper color, brown-orange. The mixture with cinchonine yields, especially with reagent C-1, fairly large branching groups of square-cut plate crystals, brown by ordinary light, noticeably but not extremely dichroic by polarized light, brownish yellow to red-brown (Figure 4, right). These predominate with 40 to 80 per cent cinchonidine, 60 to 20 per cent cinchonine. When numerous and comparatively small they may form mostly as roundish plates.

An inert substance such as lactose does not interfere with crystallization, but may obscure the crystals by particles that do not dissolve readily. Each of the alkaloids discussed was found easy enough to identify in 5 per cent mixture with 95 per cent of lactose.

Relative Value of Three Reagents

In the case of quinine the three reagents were tried out on the sulfate, bisulfate, and hydrochloride, as well as on the free alkaloid.

Iodine Reagent Q is probably best for quinine, though not by any wide margin. It is also excellent for quinidine, but cannot be satisfactorily used for cinchonine or cinchonidine.

Iodine Reagent C-1 is the best all-round reagent for the four alkaloids, and gives excellent crystals with each. It is best for cinchonine; better than reagent C-2 and possibly best for quinidine; and better than reagent Q and possibly best of the three for cinchonidine. It is also best for the mixed crystals of cinchonidine and cinchonine.

Iodine Reagent C-2 is chiefly of interest and value for its cinchonidine plate crystals which show extreme dichroism. It gives excellent quinine crystals and can be used for cinchonine and quinidine.

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PHOTOMICROGRAPHS by John B. Dalton, Police Department, Saint Paul, Minn.

MODERN LABORATORIES

The New Missouri Highway Laboratory Building

VICTOR H. LYON, Missouri State Highway Department, Jefferson City, Mo.

THE Missouri State Highway Commission has maintained a laboratory in Jefferson City since 1922 for conducting tests to control the quality of materials entering highway construction and conducting research and work of investigational nature. The first laboratory was established in the basement of the State Capitol. Later, in 1928, it was transferred to the basement of the Highway Building which had just been completed. These quarters, considered ample at the time, eventually proved inadequate, owing to the increased demands for testing and research in connec-

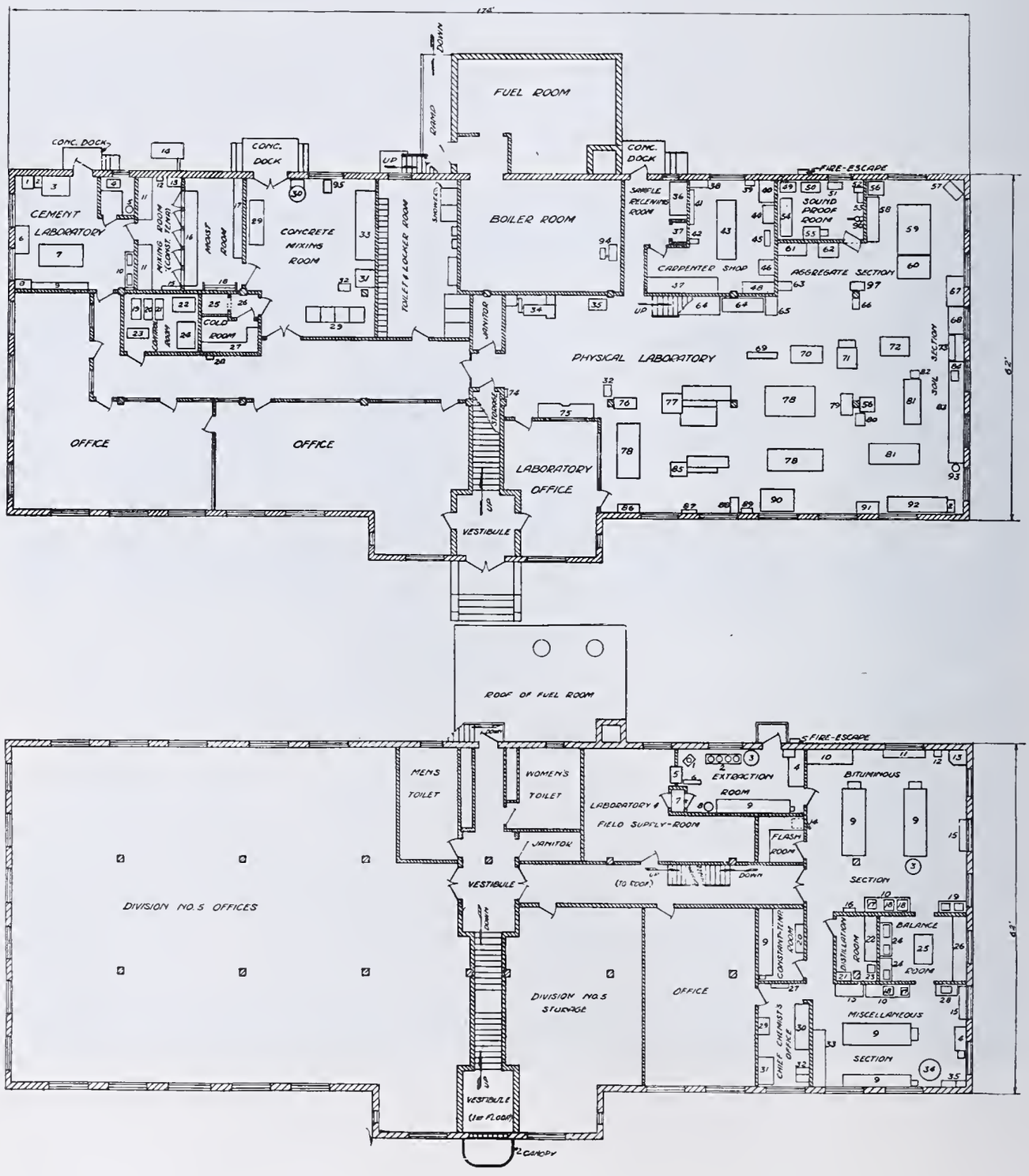
(*Upper left*) Part of Bituminous Testing Section, Flash Room at Left, and Entrance to Extraction Room
(*Lower left*) Galvanized Metal Racks in Moist Room
(*Upper right*) Machine for Cutting Cubes from Ledge Stone Samples for Abrasion Test
(*Lower right*) Miscellaneous Testing Section

tion with continued development of the highway system and advancement of construction practice. To provide for the increased needs of the laboratory, the Highway Commission authorized the construction of a building specifically designed for laboratory use. This building, financed in part by a PWA grant, has been completed and is now occupied.

The new laboratory building is a two-story structure, 62 feet wide by 174 feet long, and is of steel and concrete construction with brick walls and partitions. The exterior walls are buff-colored face brick trimmed with limestone. Large steel-sash windows provide ample light and ventilation. The building is completely equipped for the physical and chemical testing of all highway materials and for the continuance of research and investigational work.

The chemical laboratory, in which chemical analyses on various materials are conducted, is located on the second floor. It is partitioned into rooms, each of which is equipped for testing specific types of materials. One large room, approximately 30 feet square, is utilized for testing bituminous materials. A smaller room, used for testing miscellaneous materials, connects with the bituminous section by means of an intermediate room equipped with balances and a titration desk. This arrangement makes the analytical balances and titration desk readily accessible to the operators in both sections. The three analytical balances are indirectly lighted and both natural and artificial light are available on the titration desk. Water-shower sprays with pull chains are installed in the two larger rooms as a safety feature in case the clothing of any operator becomes ignited.

There are separate rooms for gasoline testing, flash and fire determinations, and extractions, and a constant-tempera-



ture room with controlled humidity. The constant temperature in this room is maintained with a Fairbanks-Morse 2-horsepower Model FQ 200W compressor which is located in the control room on the first floor. A rheostat is provided for the lights in the flash room, so that they may be dimmed to determine flash points more accurately.

The fumes from the various hoods in the chemical laboratory are exhausted by three blowers located in a penthouse on the roof. To compensate for this exhausted air during the winter months, two Nesbit Syncretized air heaters are provided to pump outside air into the rooms from grilled exterior openings, conditioning it to room temperature.

Chemical desks are supplied with all utilities, including natural gas, 110- and 220-volt electricity, compressed air, and steam. All drains are constructed of acid-resistant metal.

The cement laboratory, in which the physical tests of cement are made, is located on the east side of the first floor. It consists of a general room, a constant-temperature room with controlled humidity, and a small room with heat-exhaust facilities for housing the steam pat boiler and autoclave. The general room contains the cement briquet-testing machines and Wagner turbidimeter and is equipped with tables, balances, and the necessary equipment for weighing the batches which are transferred to the constant-temperature room for mixing.

The constant-temperature room conditions are maintained by a Fairbanks-Morse unit, using a 3-horsepower Model FQ 300W compressor located in the control room. A series of cabinet doors opens from the constant-temperature room to the moist room, which is provided with galvanized metal racks for holding test specimens.

The moist room measures 10.5 by 19 feet and is insulated with 2 inches of cork on the walls and ceiling. It is designed with an entrance on the opposite side from the constant-temperature cement-mixing room and thus serves the double purpose of curing concrete as well as cement paste and mortar specimens. The room is conditioned by pumping air charged with moisture from a conditioning unit located in the controll room into a copper duct fitted with three grille openings inside the moist room. Extra water sprays are installed in the duct to promote additional humidity. Temperature balance within the moist air-conditioning unit is provided by hot water and a Fairbanks-Morse 3-horsepower Model FQ 300W compressor. The temperature in the moist room is maintained at 70° F. and the relative humidity at 96+ per cent continuously. A TAG recording psychrometer is used to keep a constant record of the conditions in the room.

A cold room is provided for conducting freezing tests on aggregates, concrete, and other materials. This room measures 9 by 9 feet, including a vestibule and brine specimen tank. It is insulated with 8 inches of cork on the walls, ceiling, and floor with 4 inches of concrete laid on the floor insulation. The room is designed with copper ceiling coils and steel rack coils on which specimens may be placed for freezing.

The brine specimen tank is insulated from the room and is reached from the end of the vestibule. This tank measures 2 feet 6 inches by 3 feet 4 inches by 1 foot 10 inches in height, inside dimensions. The brine is refrigerated by means of coils in an overhead 200-gallon reserve tank and flows to the specimen tank by gravity with a safety shut-off valve to stop the flow when the specimen tank is filled. A motor-driven centrifugal pump, which is operated by means of an automatic temperature controller, returns the brine to the reserve tank, making the flow continuous when the temperature of the brine in the specimen tank rises above the controller setting.

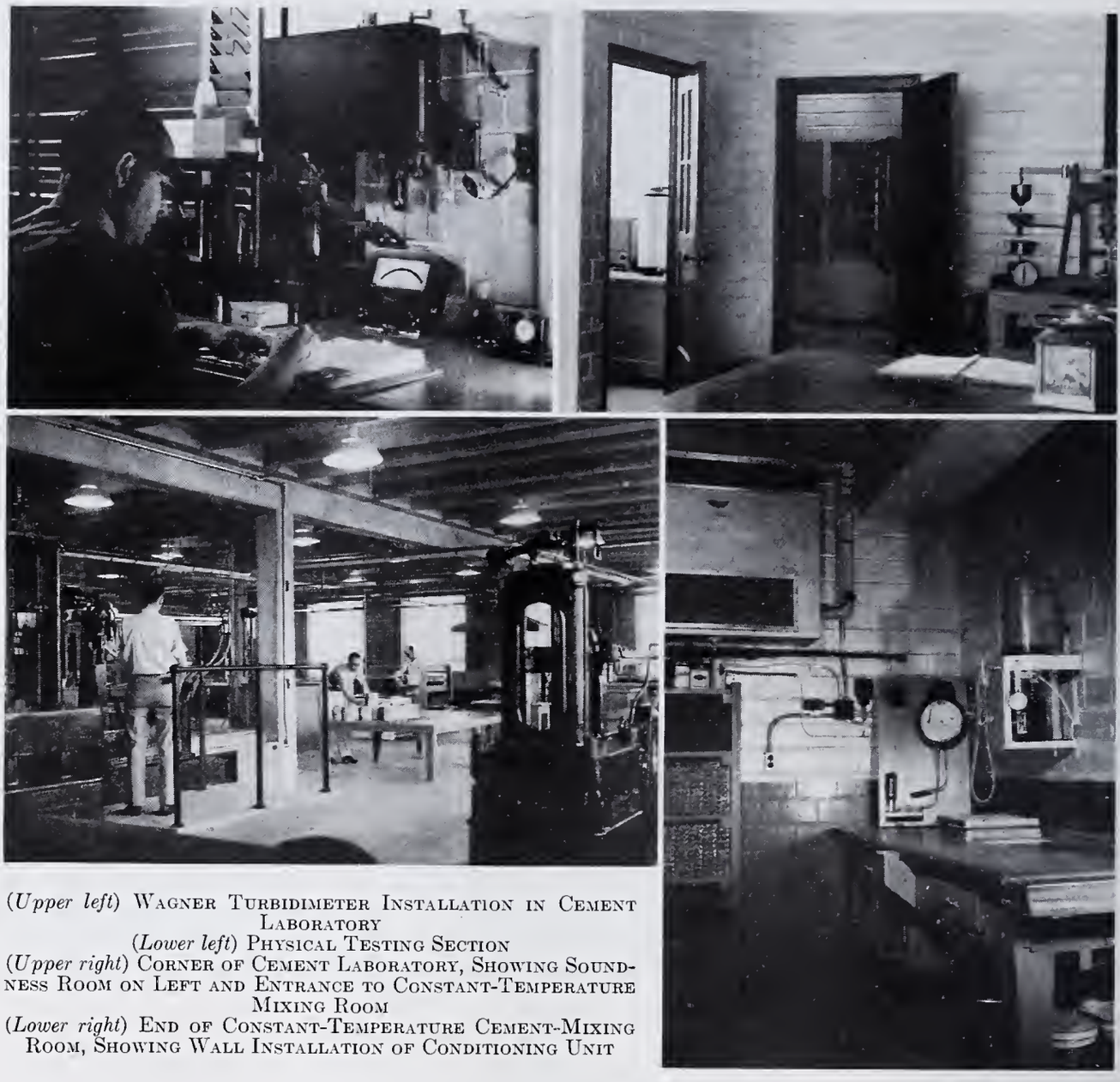
A Fairbanks-Morse 10-horsepower 2-speed Model FV 1000W compressor is used for refrigerating the cold room and brine. The two sets of coils, from the one compressor, to the cold room and brine reserve tank are operated either independently or simultaneously by means of separate solenoid valves, each having its own temperature controller. The capacity of the freezing equipment is such that the tem-

FIRST FLOOR PLAN (*Opposite Page, Above*)

1. Kerosene supply
2. File cabinet
3. Desk
4. Pat boiler
5. Autoclave
6. Turbidimeter
7. Sample preparation table
8. Storage cabinet
9. Sample shelves
10. Briquet machines
11. Mixing table
12. Recording psychrometer
13. Sink
14. Water-cooling tower
15. Mold rack
16. Briquet rack
17. Specimen rack
18. Concrete cylinder rack
19. 2nd floor constant temperature room compressor
20. Cement constant temperature room compressor
21. Moist room compressor
22. Moist room conditioning unit
23. Water pump
24. Cold room compressor
25. Brine tank
26. Cold room vestibule
27. Specimen rack coils
28. 10-Point temperature recorder
29. Thawing tanks
30. Concrete mixer
31. Sonic apparatus
32. 250-lb. platform scales
33. Cylinder capping table
34. Carborundum saw
35. Steel bending machine
36. Sample table
37. Freight elevator
38. Paint cabinet
39. Tool grinder
40. Supply cabinet
41. Wall tool cabinet
42. Drill press
43. Carpenter bench
44. Tool chest
45. Grind stone
46. Utility table
47. Lumber rack
48. Waste material
49. Ball mill
50. Rotap
51. Grinding lap
52. Jaw crusher
53. Burr mill
54. DeVal abrasion machine
55. Los Angeles abrasion machine
56. Drying oven
57. Dorry hardness machine
58. Sample cabinet
59. Aggregate screening table
60. Soil preparation table
61. Sink
62. Sand preparation table
63. Diamond drill
64. General storage cabinet
65. 500-lb. platform scales
66. Page impact machine
67 Sink
68. Desk
69. Bituminous compaction machine
70. Equipment cabinet
71. Freezing cabinet
72. Soaking tanks
73. Water bath for hydrometer test
74. First-aid cabinet
75. Brinell hardness machine
76. Testing machine accessory cabinet
77. 200,000-lb. Universal testing machine
78. General purpose table
79. Hot-plate table
80. Asphalt stability machine
81. Soils table
82. Soil compacting block
83. Soils bench
84. Stoneware sink
85. 100,000-lb. Universal testing machine
86. Testing machine accessory cabinet
87. Wall tool box
88. Strip tester accessory cabinet
89. Scott strip tester
90. Drafting table
91. Calculator table
92. Three-section desk
93. Centrifuge
94. Air compressors
95. Concrete saw
96. Soil pulverizer
97. Stone-cutting machine

SECOND FLOOR PLAN (*Opposite Page, Below*)

1. Rotarex
2. Bituminous extractors
3. Waste container
4. Wash bench
5. Service door and shelf
6. Culvert metal shears
7. Freight elevator
8. Centrifuge
9. Chemical desk
10. Hood
11. Ductility machine
12. Penetrometer
13. Constant temperature bath
14. Wall safe
15. Nesbit Syncretized air heater
16. Sample rack
17. Muffle
18. Hot plate
19. Loss ovens
20. Paint drying rack
21. Steel sample cabinet
22. Steel work table
23. Stone sink
24. Balances
25. Utility cabinet
26. Titration table
27. Book case
28. Drying oven
29. Calculator table
30. Three-section desk
31. Desk
32. File cabinets
33. Chemical supply cabinet
34. Rotating filtration table
35. Water still



(*Upper left*) Wagner Turbidimeter Installation in Cement Laboratory
(*Lower left*) Physical Testing Section
(*Upper right*) Corner of Cement Laboratory, Showing Soundness Room on Left and Entrance to Constant-Temperature Mixing Room
(*Lower right*) End of Constant-Temperature Cement-Mixing Room, Showing Wall Installation of Conditioning Unit

perature of 30 cubic feet of concrete can be lowered from 80° to −10° F. in air in 20 hours and held indefinitely at this temperature, or 6 cubic feet of concrete can be lowered from 80° to −20° F. in 8 hours in the brine and held indefinitely at this temperature. Both conditions can be met simultaneously, using 24 cubic feet in air and 6 cubic feet in the brine or a total charge of 30 cubic feet.

The concrete-mixing room is used principally for proportioning, mixing, and molding concrete test specimens for investigational purposes. A Model SW Lancaster laboratory mixer is available, as well as a series of mechanically agitated metal thawing tanks. Apparatus for the sonic measurement of modulus of elasticity and a high-speed Felker Di-Met concrete saw are located in this room.

The front half of the east wing on the first floor is partitioned into offices which are occupied by several departments of the Materials Bureau, as is another office on the second floor adjacent to the chief chemist's office. The laboratory office is located just west of the front entrance on the first floor.

The entire west wing of the first floor comprises the physical laboratory, which includes the soils and aggregate sections. Two Riehle Universal testing machines are located in this section, with other items of equipment for the physical testing of materials. Two large gas-fired drying ovens are provided for drying soil and aggregate samples, and the fumes and heat from these ovens and other drying equipment are exhausted by forced draft. A soundproof room houses the equipment which necessarily is noisy in operation, such as the rattlers, grinding lap, rotap, ball mill, crusher, and grinder.

A carpenter shop provides the necessary facilities incident to laboratory needs. No machine shop was included in the new laboratory building, as the Highway Department maintains a fully equipped shop in the headquarters garage which is available to the laboratory.

Adjoining the main building is a one-story maintenance building of similar exterior design, a section of which is provided for the laboratory. This space is partitioned to make three separate rooms, in one of which an octane machine, for determining the antiknock characteristics of gasoline, is located. This machine, a Waukesha ASTM-CFR engine, was located here, rather than in the main building, because of the noise and vibration during operation. The other two rooms are used for chemical storage and as a garage for the laboratory truck.

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Determination of Moisture in Sugar Products[1]

Filter Paper Method for Corn Sirup

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PROBABLY no class of products has created so much controversy over moisture content as the one containing sugars. Not only is the stability of the sugars themselves involved (with respect to temperature) but sirups and impure sugars contain residual acids, proteins, gums, and mineral matter which complicate the usual problem of drying, so that peculiar results are often attributed to decomposition when the variable involved is in an improper method. The problem is an old one and the numerous papers which have been published give ample evidence of the fact that it is an analytical problem in all laboratories dealing with sugar products.

The usual variables in past papers which have been published on this subject are: (1) temperature, and (2) extenuated drying surface.

Temperature is not only an important variable with respect to the stability of the sugars themselves, but becomes more significant when impurities are present. The usual safeguard is to use temperatures under 100° C. However, this prolongs the period of drying, which is critical in that many products which are comparatively stable over a given period of heat treatment develop instability over prolonged heating. In order to offset the lower temperature, vacuum ovens are employed to speed the removal of water.

Necessity for dispersion of the sample in order to provide an extenuated surface for drying, particularly in the case of sugar products, has long been recognized, the primary purpose being to speed and to ensure the completeness of moisture removal.

Carr and Sanborn (*3*) in 1895 employed pumice and their method is essentially the A. O. A. C. method of today. Rolfe and Faxon (*9*) over 40 years ago suggested paper rolls, but the method never gained acceptance, probably because of insufficient interest at that time. More recently, Rice and Boleracki (*8*) proposed the use of two silver plates to increase the surface area of the sample. Although extending the surface area, this method failed when applied to corn sirup, because on drying, the dextrin in the corn sirup formed flakes that tended to curl and "dust off" from the silver surface.

In the A. O. A. C. method, employing sand, wherein a 1- or 2-gram sample is used, weight constancy is never obtained. In order to have some criterion by which moisture determinations can be discontinued, drying is allowed to proceed "until the change in weight does not exceed 2 mg. per interval", with a 2-hour drying period in general acceptance. Such a criterion gives a precision no greater than 0.2 per cent, which becomes of considerable magnitude in the corn products industry, which sells millions of pounds of corn sirup and crude sugar annually. This criterion is also particularly unfortunate, in that the looseness of the definition of constancy has given use to many statements of decomposition and instability of the product when the essential point has been the inadequacy of the method for that particular product. As a natural result of this, research has been stopped on more adequate methods for determination of true moisture.

Rice (*7*) departed from the oven methods for sugar products by applying to refiner's sirup Bidwell and Sterling's (*2*) modification of the distillation method of Dean and Stark (*4*). He changed their technique by introducing Filter-Cel in the flask as a bed for the sample of refiner's sirup. When the distillation was started, the refiner's sirup spread through the Filter-Cel, thus combining the sample dispersion found necessary in the oven methods to a method yielding measured water. The present authors (*6*) applied Rice's distillation technique to corn sirup and found that the corn sirup did not spread but formed a hard ball, necessitating the incorporation of the sample manually in the Filter-Cel before distillation was started. This work with tables for Baumé-moisture values has been published (*5*); it gave moisture values 0.7 to 1.8 per cent higher than values by the usual A. O. A. C. sand method.

From the standpoint of a further reduction in losses, this discrepancy became of enormous importance to the corn products industry wherein factory yield is determined from incoming and outgoing dry substance. As a result, for the past 8 years an extended investigation of true moisture has been carried out on the products of the industry—corn sirup, crude sugar, steep water, feeds, hydrol—with the refinement of old moisture methods and the development of new drying technique. These have such general interests that they have been extended to other sugar products. This work on analytical methods for true moisture will be recorded in a series of papers, of which this is the first.

Corn Sirup and Corn Sugar

Corn sirup is the thick viscous sirup obtained from the partial hydrolysis of starch. It is a mixture of carbohydrates and, in addition, contains small amounts of residual acidity, crude protein, and ash. Corn sirup is of several types, and the degree of hydrolysis is defined by the amount of reducing sugars expressed as dextrose on a dry-substance basis, to which the expression "dextrose equivalent", or simply D. E., has been assigned by the industry. The authors' average analysis of 42 dextrose equivalent corn sirup, the commonest

[1] This is the first article in a series on this subject. The second follows on page 858.

type, known generally as confectioners' sirup, is as follows on a dry-substance basis:

Reducing sugars as dextrose	42
Dextrin	37
Maltotetrose	5
Maltotriose	15
Maltose	21
Dextrose	22
Residual acidity as hydrochloric acid	0.02
pH, 50% by weight	5.0
Ash	0.28
Crude protein	0.05

The dextrin content of corn sirup makes a moisture test by the A. O. A. C. method difficult, as the sirup does not spread easily, tends to form a skin by surface drying, and sets to a concretelike mass from which the final traces of moisture are removed with difficulty or not at all. The presence of ash, residual acidity, and protein, particularly since dextrin is present, has always cast serious doubts as to whether the slowly removed last traces of moisture were moisture of the sirup or water of decomposition.

FIGURE 1

Crude corn sugar, more familiarly known in the trade as "70" and "80" sugars, may be said to represent the complete acid hydrolysis of starch in commercial practice. The product cannot be so closely defined as corn sirup because of trade uses, but the composition may be expressed as follows:

Reducing sugars as dextrose	82 to 92
Acidity	0.30 to 0.40
pH, 50% solids	4.2 to 5.2
Ash	0.6 to 1.25
Crude protein	0.08 to 0.20

There is little if any dextrin in crude sugar, so the drying problem differs from that of corn sirup, but it is further complicated by the larger percentage of reactive dextrose and by the increasing quantities of residual acid, ash, and protein.

The method of the present authors, employing toluene distillation, has a decided advantage over the A. O. A. C. sand method, as the size of the sample used is tenfold, increasing the accuracy of the test. The higher values obtained for corn sirup by this method as compared to the A. O. A. C. method evoked criticism of decomposition because of the higher temperature involved (110° to 112° C.), despite the fact that the values for corn sugar were in relatively close agreement with those of the A. O. A. C. method. It would normally be thought that corn sugar would be more susceptible to decomposition because of the larger percentage of dextrose and residual traces of acidity, ash, and protein. As a result, the initial stages of this investigation of true moisture center on comparison of the data by toluene distillation with the A. O. A. C. method at 70° and 100° C., together with new technique at these temperatures.

Method by Drying on Sand

The investigation of moisture determination in corn sirup by drying on sand was carried out in a vacuum oven at 74-mm. (mercury) pressure and in an air oven. The temperatures in the vacuum oven were 70° C., conforming to the A. O. A. C. (*1*), and 100° C., a practice that has become part of the routine analytical procedure in the corn products industries. The air oven temperature was 100° C.

Acid-washed quartz sand was used. It was screened for size, that passing a 20-mesh and retained on a 40-mesh being used. Approximately 35 to 40 grams were run into an aluminum dish, 7.5 cm. (3 inches) in diameter. Original weights included a small pestle and a cover. Immediately on removing the test from the oven for cooling in a desiccator, the cover was placed in position, as it was found that the material was hygroscopic. Dishes, sand, and pestle were dried in an air oven at 100° C. overnight and removed to a desiccator the following morning previous to use.

The corn sirup was added to the sand in two ways:

1. According to the A. O. A. C. method (*1*). A sample of sirup was diluted to a known volume, and from it was pipetted a known quantity containing approximately 1 gram of dry substance which was added to the sand in the dish. The dish was then placed on a steam bath in order to remove the excess water, and the contents were stirred with the pestle until the sand became stiff. It was then removed to a vacuum oven and dried to constant weight.
2. Direct addition to the sand. The approximate amount of sirup was run on the sand in a dish previously weighed, and then reweighed. The dish was placed in the oven for approximately 15 minutes, after which it was removed and the sirup was thoroughly incorporated with the sand. The dish was then returned. At the end of 4 hours, the first weight was made for moisture loss and thereafter periodically every 2 hours until the loss was nearly constant, after which a longer interval was taken.

A large quantity of corn sirup was obtained and run into 118-ml. (4-ounce) wide-mouthed screw-capped bottles, each bottle being used for a set of tests and discarded, thus eliminating the possibility of moisture loss by evaporation of the stock material. The dextrose equivalent of the sirup was 43.1 with acidity, ash, and protein as above.

The data in Table I show that the moisture by air oven at 100° C. is less than that obtained in a vacuum oven at the same temperature, but equal to or greater than that obtained by the A. O. A. C. procedure for incorporation with the sand employing vacuum at 70° C. The vacuum oven at 100° C., with direct incorporation of the sirup with the sand, gave results at the end of 20 hours equal to those obtained by toluene distillation. The higher results obtained by this method are attributed to the greater surface area produced by stirring the hot sirup in the sand. The resulting material was furrowed and comparatively loose, this structure being maintained during the drying process. Twenty hours, the time necessary to secure a constant weight, is much too long for laboratory procedure, and it was thought that if a greater effective surface area could be produced, a moisture determination could be

TABLE I. MOISTURE OF CORN SIRUP BY DRYING ON SAND

(Moisture by toluene distillation, 19.30%)

Type of Oven	Temperature of Oven	Approximate Weight of Sample	Method of Adding Sirup to Sand	Moisture					
				4 hours	6 hours	8 hours	10 hours	12 hours	20 hours
	° C.	Grams		%	%	%	%	%	%
Vacuum	70	1	A. O. A. C.	17.22	17.57	17.85	17.96	18.07	18.41
	70	1	A. O. A. C.	17.64	17.80	18.01	18.03	18.04	18.39
	100	2	Direct	19.06	19.10	19.22	19.25	19.27	19.27
	100	2	Direct	19.13	19.21	19.27	19.28	19.29	19.31
Air	100	1	Direct	17.62	18.07	18.43	18.63	18.73	18.81
	100	1	Direct	18.56	18.65	18.79	18.84	18.91	18.97
	100	2	Direct	17.77	18.00	18.10	18.17	18.24	18.36
	100	2	Direct	17.80	18.04	18.12	18.25	18.28	18.36

Table II. Moisture of Corn Sirup by Drying on Filter Paper

(Moisture by toluene distillation, 19.30%. Temperature of oven, 100° C.)

Type of Oven	Approximate Weight of Sample	Weight of Water Added	Area of Filter Paper	Moisture 4 hours	6 hours	8 hours	10 hours	20 hours
	Grams	*Grams*	*Sq. in.*	%	%	%	%	%
Vacuum	1	1	20	18.75	18.97	19.09	19.13	19.26
	1	1	40	19.07	19.16	19.21	19.22	19.25
	1	1	60	19.14	19.20	19.24	19.26	19.29
	1	1	70	19.15	19.26	19.28	...	19.31
	1	2	70	19.20	19.30	19.30	19.30	19.31
	2	2	40	18.93	19.06	19.14	19.15	19.18
	2	2	60	19.03	19.16	19.17	...	19.28
	2	2	70	19.05	19.19	19.24	19.26	19.29
Air	1	1	40	18.67	18.92	19.01	19.03	19.23
	1	1	60	18.82	19.05	19.08	19.08	19.29
	1	1	70	19.00	19.10	19.18	19.22	19.28
	1	2	70	19.10	19.19	19.24	19.25	19.29
	2	2	70	18.30	18.42	18.50	18.54	19.07

made on corn sirup within 6 or 8 hours, which also would remove the criticism that decomposition occurs through prolonged heating.

Moisture by Drying on Filter Paper

During the investigation for such a condition, the authors considered various proposals but finally settled on one which has given highly satisfactory results.

The sirup was diluted and dried on filter-paper coils (Figure 1), held together with a paper clip. The coils were made by rolling a strip of filter paper onto a corrugated phosphor-bronze separator, the paper being longer and wider than the separator, in order that the ends and the outer layers of the coils might be made of filter paper. The filter paper must show a pH of less than 7 when an indicator is applied to it. This precaution is sufficient when corn sirup is dried, since this material contains a small amount of residual acid and is fairly well buffered, but when pure sugar solutions are dried, it often becomes necessary to acidify the solution slightly with an acid such as lactic in order to prevent decomposition. Several types of filter paper were examined and Whatman No. 1 proved to be the most satisfactory.

Apparatus. Filter paper. A strip of crepe filter paper 4.375 × 50 cm. (1.75 × 20 inches) with a pH of less than 7.

Separator. Corrugated strip of phosphor-bronze 1.25 × 40 cm. (0.5 × 16 inches) No. 36 B. & S., made by running the original strip through small-size meshed gears.

Weighing bottle. Medium form, 40 × 65 mm. with a 40/20 standard-taper ground-glass stopper.

Paper clip. Common type, to hold the filter paper in position with reference to the metal separator.

Procedure. A paper coil and weighing bottle were dried in an air oven at 100° C. for 6 hours, cooled in a desiccator, and weighed. The weighing bottle must always be closed with its stopper before it is removed from the oven to the desiccator, in order to prevent the adsorption of moisture not only by the filter paper itself but also when it is covered by corn sirup, which is quite hygroscopic. The coil was removed, approximately 1 gram of sirup was run into the weighing bottle, and the exact weight was obtained. To this sirup was added 1 or 2 ml. of distilled water; the bottle was warmed, and the water and sirup were mixed to form a dilute solution. The paper coil was then introduced and allowed to absorb the sirup solution. The last trace was taken up by shaking the coil across the bottom of the weighing bottle. The weighing bottle should not be placed in the oven until the filter paper has thoroughly absorbed the sirup, as evidenced by the disappearance of excess sirup where the edge of the filter paper touches the bottom of the weighing bottle.

The steps in the determination of the proper amount of sirup for filter paper are shown in Table II. In computing the area of the filter paper, both sides are counted as effective.

Table II shows clearly that as the area of the filter paper per gram of sirup increases, the time for effecting a moisture determination correspondingly decreases. With 451 sq. cm. (70 sq. inches) and 1 gram of sirup, the time for a test should not exceed 6 hours in a vacuum oven at 100° C. Again, the moisture content is found to be equal to that obtained by toluene distillation.

The above procedures were applied to "70" sugar. The dextrose equivalent of the sample used was 80.8; otherwise the sugar conformed to the general specifications above. The data obtained are similar to those for corn sirup and are found in Table III.

Conclusions

The A. O. A. C. method does not seem particularly adapted to corn sirup and sugar for accurate work.

The usual moisture determinations of corn sirup and sugar on sand in an air oven are invariably low.

Moisture determinations of corn sirup and corn sugar on sand in a vacuum oven can be made satisfactorily, provided sufficient time is taken and the ratio of sample to sand is correct. The latter involves dish size.

Moisture determinations of corn sirup and corn sugar by means of the proposed filter paper method are more accurate and much more rapid.

Moisture determinations on corn sirup by the toluene distillation method, although higher than the A. O. A. C. method, have the same precision as vacuum oven methods at 100° C., provided the latter are carried out under conditions adaptable to the viscous characteristics of corn sirup. However, both these methods are subject to the general criticism that both temperatures might produce decomposition, which could be avoided at lower temperatures.

Table III. Moisture of Corn Sugar

(Moisture by toluene distillation, 17.45%. Vacuum oven used)

Temperature of Oven	Approximate Weight of Sample	Method of Adding Sirup to Sand	Weight of Water Added	Area of Filter Paper	Moisture 4 hours	6 hours	8 hours	10 hours	20 hours
° *C.*	*Grams*		*Grams*	*Sq. in.*	%	%	%	%	%
		By Drying on Sand							
70	1	A. O. A. C.			17.00	17.11	17.14	17.15	17.30
	1	A. O. A. C.			16.90	17.02	17.08	17.11	17.22
100	2	Direct			17.25	17.28	...	17.30	17.42
	2	Direct			17.30	17.31	...	17.31	17.38
		By Drying on Filter Paper							
100	1		2	70	17.36	17.44	17.44	17.45	17.45
	1		2	70	17.37	17.40	17.42	17.44	17.44

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Determination of Moisture in Sugar Products

Use of Filter-Cel for Corn Sirup

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IN THE belief that the controversy over the moisture content of corn sirup would never be settled until lower temperatures were used, which in turn would require a more complete sample dispersion or a still thinner surface film for drying than was obtained in the filter paper method, it was decided to attempt to apply Filter-Cel, used in the toluene distillation as a dispersing medium, to a straight vacuum-oven method, thereby not only obtaining a very large surface area but also employing a larger sample weight. The technique proved unusually successful, and since the procedure has been adopted as official for the corn products industry by the Technical Advisory Committee of the Corn Industries Research Foundation, it is described here in detail.

TABLE I. DATA ON CORN SIRUP

Time	Moisture Found at 100° C. 10.6382-gram sample	10.0545-gram sample
Hours	%	%
8	14.89	14.87
18	14.98	15.03
39	14.99	15.02
	At 60° C.	
	10.5626-gram sample	10.6021-gram sample
15	14.55	14.54
38	14.88	14.89
60	14.96	14.92
100	14.98	14.96

Materials and Apparatus

FILTER-CEL. A large quantity of Filter-Cel is washed by percolation with distilled water that has been slightly acidulated with hydrochloric acid. This treatment is continued until the effluent is acid to litmus. Washing with distilled water follows until the effluent is essentially neutral, and the Filter-Cel is then air-dried. A quantity, usually a quart, is transferred to an air oven at 105° C. and kept for use.

SAMPLE CONTAINERS. For the determination, two types of apparatus are used—one for referee samples, or those requiring the highest degree of precision, and one for factory or routine procedure.

REFEREE SAMPLES. A 250-ml. Pyrex Erlenmeyer or wide-mouthed Erlenmeyer flask, neck ground to 40/50 standard taper is used. This is the same flask that is used for distillation tests. The closure is a 40/12 standard-taper weighing bottle stopper.

ROUTINE SAMPLES. A 7.5-cm. (3-inch) aluminum dish with deep slip cover, which is sold as a small crucible desiccator, or 1-pound friction-top tins in use in corn products plants for packaging of mixed table sirups are used.

STIRRER. The stirrers or pestles are 100 × 15 mm. Pyrex test tubes. The stirrer extension is a glass rod, fitted at one end with two rubber rings, cut from tubing and so spaced that when inserted into the test tube a rather snug fit is obtained at top and bottom of tube. The rod, with its glass test tube end, makes an easily manipulated stirrer. The rod is removed after the stirring operation, leaving the test tube with the Filter-Cel sample mass.

Procedure

Filter-Cel (25 grams) is run in duplicate flasks, the test tube is added, and the stopper is set at a 90° angle in the mouth of the flask, which is brought to constant weight in the vacuum oven at the temperature at which the drying test is to be carried out. A third flask, with stopper only, is used as a tare in weighing and put through the same procedure as flasks used for samples.

Because of surface moisture adsorption, it was found desirable to use individual desiccators: 5- or 10-pound friction-top cans, commonly used for packaging mixed table sirup. The desiccant is phosphorus pentoxide.

The sample weight always is taken so that 5 to 8 grams of solids result. Samples below 35° Bé. are handled by means of a pipet (from which the tip has been cut off) by running approximately 20 to 40 ml. of solution on the Filter-Cel, stoppering, and reweighing for sample weight. For samples over 35° Bé., 8 to 10 grams are weighed in a nickel scoop, to which 10 ml. of distilled water are added. The scoop is warmed on a steam bath to facilitate the formation of a homogeneous sirup, which is run onto the Filter-Cel. The scoop is washed successively with three 5-ml. portions of distilled water. The Filter-Cel–sirup mixture is then gently worked to a damp mass. This is an important step, as the ratio of Filter-Cel to sample plus washings must always yield a damp mass, so that the Filter-Cel retains its powdered form. A wet mass will produce a concretelike structure after drying.

In order to remove the excess water, the flasks are then placed in a vacuum oven maintained at a pressure of 50 to 75 mm. by a water pump. This usually requires 2 to 4 hours. The flasks are removed, the Filter-Cel mass is quickly reworked to a powder, and the flasks are replaced in the vacuum oven, this time actuated by a Hyvac or Megavac pump. It is important to rework at an early stage, as the mass is then very friable and easily handled, whereas it may become difficult if left until the final stage of dryness. Constant weight—i. e., within 1 mg. or 0.01 per cent—is usually obtained in 15 hours or more, depending upon the original condition of the Filter-Cel mass.

The precision by this method is surprisingly good, checks often agreeing in the second decimal place. The dispersion of the sample is so complete that lower temperatures in the vacuum oven can be used for drying, a particularly valuable technique with heat-sensitive materials. Some idea of results obtainable can be gained from experimental data (Table I) on a sample of 45° Bé. corn sirup, which is nearly a plastic at room temperature, and from which a sample can be obtained with difficulty.

The method has been in successful use in the corn products industry for 3 years.

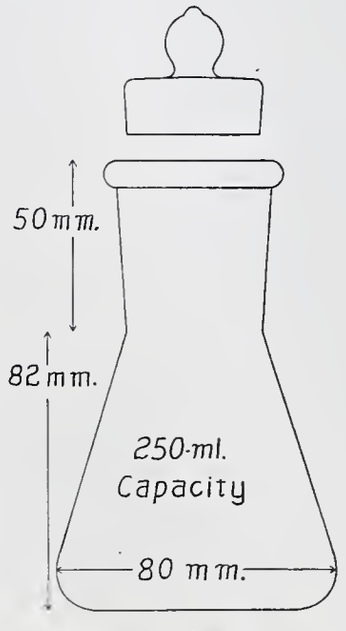

FIGURE 1. APPARATUS

Weighing bottle ground-glass stopper $\frac{S}{}$ 40/12. Standard flask used in toluene distillation method for corn sirup $\frac{S}{}$ 40/50. 15 × 100 mm. test tube

Objections to Filter-Cel

It is difficult to bring to constant weight. The material used here did not present this problem, as it had been calcined by the manufacturer and washed as described above.

It is prone to dust under vacuum or during manipulation. This was not found to be the case when used with reasonable care.

It is a good heat insulator and hence permits only a slow heat transfer to the sample. The mass is wet in the initial stages of drying and the heat transfer is good, while in the latter stages, as the Filter-

TABLE II. HYGROSCOPICITY OF FILTER-CEL

Elapsed Time, Hours	Gain in Weight at Relative Humidities of: 35%	45%	52%	66%	78%
5	0.020	0.031	0.036	0.041	0.075
15	0.028	0.041	0.041	0.058	0.085
72	0.039	0.048	0.048	0.063	0.096
119	0.060	0.071	0.079	0.096	0.12
143	0.060	0.071	0.079	0.096	0.13
167	0.060	0.071	0.079	0.096	0.13

Cel mass becomes drier, the heat transfer is slower. A maximum recording thermometer placed in the mass showed that approximately 1 hour was required for the center to reach the oven temperature, either 70° or 100° C. Pieces of fine copper wire (1.25 to 2.5 cm., 0.5 to 1 inch) incorporated with the Filter-Cel did not sufficiently improve the heat transfer to warrant their use. The heat transfer can be improved in a vacuum oven by soldering the tray to the wall support.

It is hygroscopic. The Filter-Cel used had a notable lack of hygroscopicity, despite its large surface area. This was shown by placing 10-gram samples of Filter-Cel in aluminum moisture dishes (25 × 75 mm.) and bringing them to constant weight in a vacuum oven at 100° C. The dishes were then transferred to humidity chambers with relative humidities of 35, 45, 52, 66, and 78 per cent (average temperature 30° C.). The dishes were weighed periodically, the mass being stirred after each weighing. Equilibrium was reached in 119 hours, as shown in Table II.

Adaptation of Filter-Cel Method

The Filter-Cel method can be adopted to usual routine conditions.

Ten grams of prepared Filter-Cel were run into aluminum moisture dishes (25 mm. in height, 75 mm. in diameter) and brought to constant weight. Five grams of corn sirup, weighed in a nickel scoop, were diluted with 5 ml. of distilled water and run onto the Filter-Cel. The scoop was then washed with three successive 2-ml. portions of distilled water which were added to the Filter-Cel. These proportions yielded a damp workable mass which, after thorough incorporation of sirup and Filter-Cel, was dried in the vacuum oven at 100° C.

The results in five dishes run simultaneously were as follows:

Per Cent Moisture	
42.0 dextrose equivalent	55.0 dextrose equivalent
19.46	20.48
19.46	20.49
19.44	20.50
19.49	20.47
19.44	20.49

These results show satisfactory precision. However, considerable patience and care are necessary to obtain checks within 0.1 per cent.

The success of the method at 60° C. suggested that the drying might be carried out at still lower temperatures if suitable apparatus could be devised to handle the Filter-Cel method under higher vacuums than obtainable in a vacuum oven. This evidence would provide the final proof of the stability of corn sirup at temperatures of usual moisture determinations.

The experimental work went through several stages of development. The design which has proved most successful from the standpoint of manipulation is shown in Figure 1.

A review of the literature shows that a similar problem confronted Lobry de Bruyn and Van Laent (*3*). Their apparatus was essentially two small flasks, connected by a curved glass tube employing rubber tubing joints. One flask contained the sample and the other phosphorus pentoxide. Vacuum was applied to the apparatus through a stopcock sealed in a curved tube. Brown, Morris, and Millar (*1*), Rolfe and Faxon (*4*), and later Walker (*6*) applied the device. None of these investigators had employed dispersion of sample in the use of the apparatus.

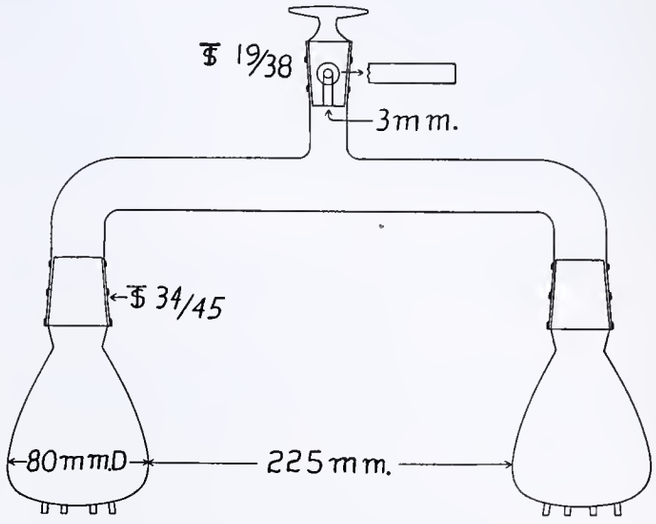

FIGURE 2. IMPROVED APPARATUS

The basic idea of the original authors was reincorporated in a new design, which made possible the application of the Filter-Cel method to the sample in one of the flasks. Several designs were tried, and from the experience gained a type was developed which has proved exceptionally easy to manipulate and, through standard tapered joints, holds high vacuums for extended periods without recourse to the vacuum pump (Figures 2 and 3).

Procedure with New Apparatus

The Filter-Cel and flasks are prepared as for the procedure at 100° C. Success in drying is obtained by having sufficient water present in the sample, so that a damp powder can be worked easily with the pestle.

The flasks containing the damp Filter-Cel mass are placed in a Weber vacuum oven at 38° C. (100° F.) which is connected, through a receiver containing a large quantity of calcium chloride, to a Hyvac pump and allowed to remain there overnight. The next morning the Filter-Cel mass in the flask is reworked to a fine powder, phosphorus pentoxide is run into the other flask, the flask joints are lubricated with a small amount of Cello grease, and the apparatus is connected to a high-vacuum pump, until the system is thoroughly pumped down. The apparatus is dis-

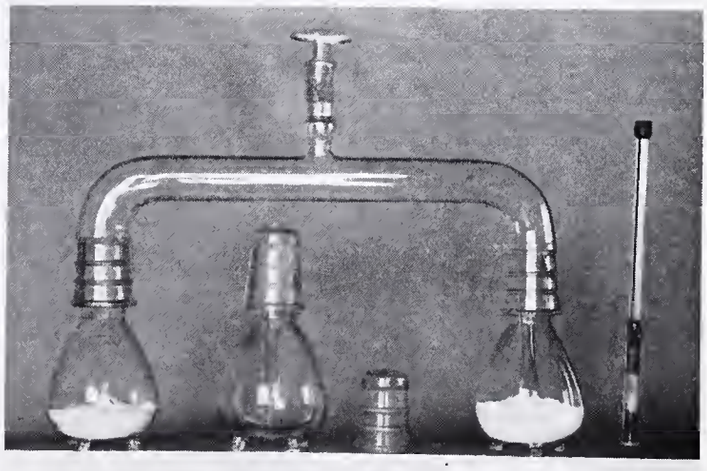

FIGURE 3. PHOTOGRAPH OF APPARATUS

connected from the pump, the flask containing the Filter-Cel mass is placed in an air bath at 37–38° C. (100° F.), and the other flask in a bath of running water for cooling, in order to maintain a temperature drop through the apparatus.

The vacuum on the apparatus is checked daily by means of a McLeod gage, and repumped when necessary. For weighing, the vacuum is released by carefully introducing air through the following train: two 19-liter (5-gallon) bottles containing about 1.25 cm. (0.5 inch) of sulfuric acid; sulfuric acid tower, fitted with fluted diffuser; tower with cotton; tower with Drierite; and large vessel fitted with trap covered with phosphorus pentoxide. Cello grease is removed from the joint of sample flask before capping by wiping with a cloth moistened in benzene.

The flasks containing the Filter-Cel mass and the phosphorus pentoxide should be rotated daily to expose a fresh surface. If the latter shows any signs of crust formation, it should be renewed.

Phosphorus pentoxide is often criticized as a desiccant (*5*), although conceded to be the most efficient of the common ones. A liter of air in equilibrium with it will contain 2×10^{-5} mg. (0.00002 mg.) of water, compared to 0.003 mg. for specially concentrated sulfuric acid or for dry ice (−70° C.). The most common criticism is that it very quickly becomes "surface sealed" by liquid acid, whereby its efficiency falls. This criticism is valid but it can be avoided if proper precautions are taken. The procedure for low-temperature drying (38° C.) recognized the desirability of maintaining a fresh surface of phosphorus pentoxide. In the first place, it was not used until evacuation had reduced the moisture as far as possible. The phosphorus pentoxide was changed frequently and between drying periods the flask was tapped or rotated so as to expose a fresh surface.

The method proved to be most successful and the technique is easily acquired. Some idea of the data obtained can be observed from Table III.

Other typical data on corn sirup, employing 100° C. in the vacuum oven by the Filter-Cel method are given in Table IV.

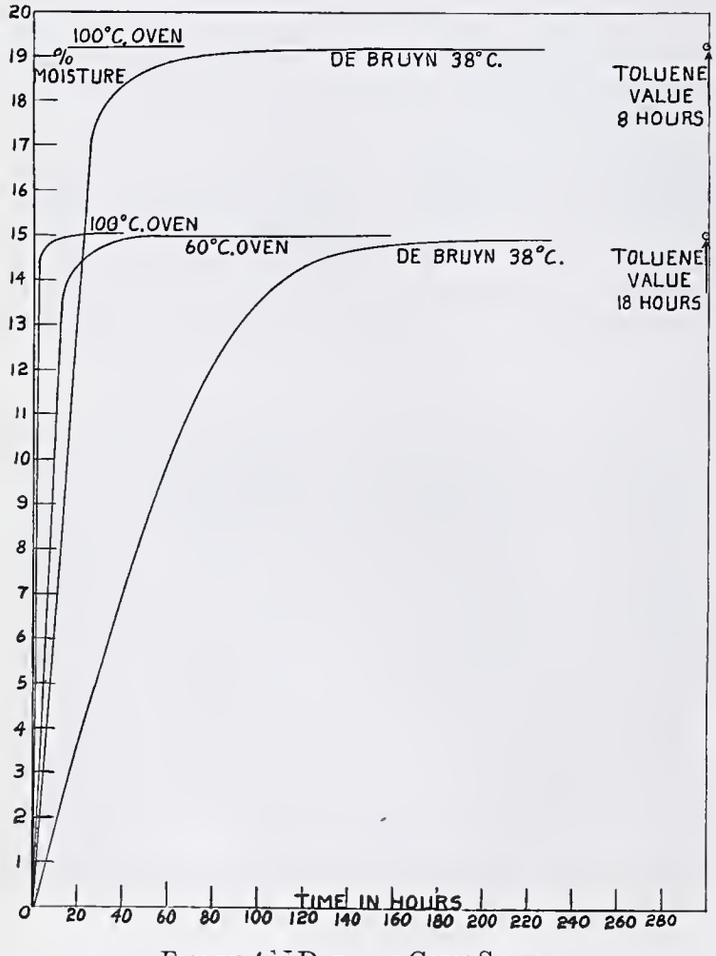

FIGURE 4. DATA ON CORN SIRUP

TABLE III. DETERMINATION OF MOISTURE

(Material, Corn Sirup, Commercial Baumé 43.22°, D. E. 42.6)

Elapsed Time	Conditions		Moisture 10.3065-gram sample	Moisture 10.1260-gram sample
Hours			%	%
	Filter-Cel with 100° C. Vacuum Oven			
6	27-inch water vacuum and sample reworked			
9	Hyvac pump		19.14	19.17
34	Hyvac pump		19.20	19.17
66	Hyvac pump		19.22	19.19
	Lobry de Bruyn–Filter-Cel Method, 38° C. (100° F.)			
				9.4712-gram sample
27	Vacuum oven			17.13
48	Lobry de Bruyn pressure	40 mm.		18.72
92	Lobry de Bruyn pressure	2 to 4 mm.		19.00
113	Lobry de Bruyn pressure	<0.1 mm.		19.14
157	Lobry de Bruyn pressure	<0.1 mm.		19.14
177	Lobry de Bruyn pressure	<0.1 mm.		19.20
222	Lobry de Bruyn pressure	<0.1 mm.		19.20
	Flask transferred to vacuum oven at 100° C.			
227	Hyvac pump			19.20
	Lobry de Bruyn–Filter-Cel Method, 38° C. (100° F.)			
				8.8289-gram sample
20	Vacuum oven			18.38
41	Lobry de Bruyn pressure	<0.2 mm.		19.02
69	Lobry de Bruyn pressure	<0.2 mm.		19.10
150	Lobry de Bruyn pressure	<0.2 mm.		19.16
216	Lobry de Bruyn pressure	<0.2 mm.		19.19
373	Lobry de Bruyn pressure	<0.1 mm.		19.22
	Flask transferred to vacuum oven at 100° C.			
378	Hyvac pump			19.22

Two further examples are shown in Figure 4, one a 43° Bé. and the other a 45° Bé. grade corn sirup.

Conclusions

New methods of dispersion of viscous sirups for standard vacuum oven procedure and of drying at low temperatures have been developed.

The stability of corn sirup at 100° C. in vacuum and at 112° C. with boiling toluene has been demonstrated by essentially identical results obtained through drying at 38° C. under low pressure.

The moisture-Baumé–dextrose equivalent relationship as shown in the original Fetzer-Evans (*2*) tables is correct.

Filter-Cel (diatomaceous earth) is an excellent medium for dispersal of viscous materials.

TABLE IV. DETERMINATION OF MOISTURE

Elapsed Time	Pressure	Sample Weight		Moisture	
Hours	*Mm.*	*Grams*	*Grams*	%	%
Commercial Baumé 39.68° (Bé. = Bé. 140°/60° F. + 1.00° Bé.), D. E. 55.0. Stream of air passing through oven, 2 hours. Small stream of dry air passing through oven, 2 hours.					
4	...	11.2014	12.2193	24.85	23.13
15	<1			25.41	25.44
22	<1			25.43	25.44
37	<1			25.45	25.45
44	<1			25.45	25.45
	Commercial Baumé 41.03°, D. E. 42.0				
4	Through calcium chloride trap, Megavac pump	12.2920	11.7820	23.48	23.40
19	<1			23.60	23.60
24	<1			23.61	23.62
39	<1			23.62	23.63

Literature Cited

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(2) Fetzer, W. R., and Evans, J. W., IND. ENG. CHEM., ANAL. ED., **7**, 41 (1935).
(3) Lobry de Bruyn, C. A., and Van Laent, F. H., *Rec. trav. chim. Pays-Bas*, **13**, 218 (1894).
(4) Rolfe, G. W., and Faxon W. A., *J. Am. Chem. Soc.*, **19**, 698 (1897).
(5) Snyder and Sullivan, IND. ENG. CHEM., **16**, 1164 (1924).
(6) Walker, P. H., *J. Am. Chem. Soc.*, **29**, 541 (1907).

Spectrochemical Analysis of Alkali Products

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THIS laboratory was installed for making daily control analyses of sodium hydroxide, soda ash, sodium bicarbonate, and sodium chloride brine. Additional quantitative analyses are made on precipitated calcium carbonate, calcium hydroxide, and calcium chloride.

The analyses use the internal control method (*3*, *4*), in which an element not already present in the solution to be analyzed is added in a fixed and constant amount and the spectral lines of this element are used for control. The relative intensities of the line of the element to be tested and a line of the control element are measured and the log ratio between them is determined. Because the control element is present in a constant amount, the ratio between the lines is a function of the test element only.

Working curves are made by plotting the log of the ratio of the line intensities against the log of the concentration of the impurity, giving a linear function.

Equipment

A Bausch & Lomb Littrow spectrograph equipped with quartz optics is used. A quartz condensing lens is placed between the light source and the slit of the spectrograph to shorten the exposure time. An image of the arc is focused on the collimator lens.

Two fixed slits (30 and 40 microns wide) mounted on a single slide are used in place of the adjustable slit on the spectrograph; because of the constant changing of the slit width, the adjustable slit on the instrument became inaccurate and required frequent checking. The slide, containing the two fixed slits, is so mounted that either slit opening can be placed directly in front of the adjustable slit, open at 1.5 mm. This wide opening of the adjustable slit is used when placing the calibrating pattern on the plate. The 40-micron slit is used for spectrograms in the ultraviolet, while the 30-micron slit is for the visible region of the spectrum.

The arc source is the high-voltage arc developed by Duffendack and Thomson (*1*), and is capable of operating up to 5 amperes at 2300 volts or 2.2 amperes at 4600 volts. A small inductance is placed in the primary side of the circuit.

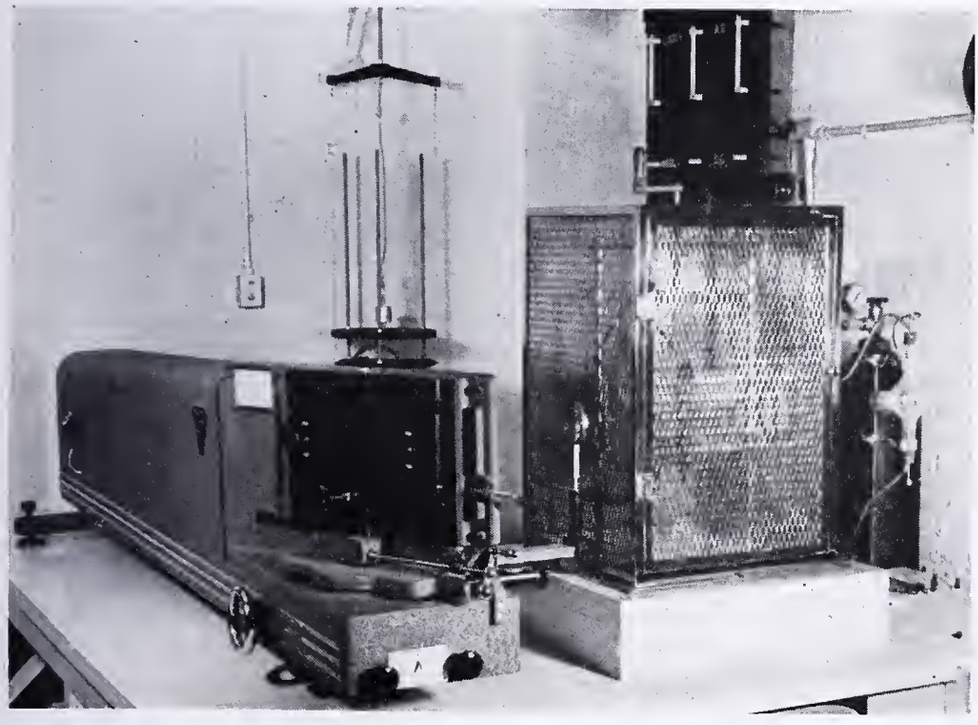

FIGURE 1

The arc stand is the same design as that used by Duffendack and Wolfe (*2*). Figure 1 shows the stand, with other parts of the source in the background. A 110-volt direct current arc is connected with the same stand and can be used in place of the alternating current source by throwing a single switch.

The photographic plates (Eastman Polychrome) are calibrated with a rotating stepped sector placed in front of the slit. The light source for the calibrating pattern is a high-voltage (20,000 volts, 25 milliamperes) spark across aluminum electrodes in a hydrogen atmosphere. This source can be seen to the right in Figure 1.

A Hilger nonrecording microphotometer with a Cambridge galvanometer is used for determining the line intensities.

Analysis of Caustic Soda

The method of analyzing sodium hydroxide is that developed by Duffendack and Wolfe (*2*), except that the 2300-volt alternating current arc operating at 5 amperes is used after preburning the coated electrodes at 2.5 amperes.

The sample to be analyzed is diluted to 25 per cent and 25 p. p. m. of molybdenum as a solution of sodium molybdate are added for an internal standard. For caustic solutions weaker than 25 per cent, the sample is diluted to 10 per cent and 10 p. p. m. of molybdenum are added. Separate sets of calibration curves are necessary for the 25 and 10 per cent solutions.

The coating and burning procedures have been described (*2*).

Calibration curves for silicon, aluminum, calcium, magnesium, iron, manganese, copper, nickel, strontium, and lead have been completed.

Because of the small amount of iron and manganese present, the faint lines are influenced by the background of the spectrum. This effect was overcome by using a faint molybdenum line (2649.5) for standardizing. In this manner both the impurity line and the molybdenum line are equally affected by the background.

In the determination of nickel and copper in sodium hydroxide, the heavy background around the principal impurity lines was again a serious problem. There is no faint molybdenum line near to calibrate against, which will correct for a change in background intensity. The changing background will shift the bottom of the calibration curve in the manner shown in Figure 2. The resulting error may be as great as 50 per cent in the range from 0.8 to 2.5 p. p. m. of nickel.

This error was overcome by determining the log ratio of the line with the background next to the line instead of using a molybdenum line. Such a calibration is used only in the lower range of analysis where a correction of background is necessary.

Preparation of Working Curves

Working curves for analysis are determined by running the prepared solutions, in which the amounts of the test elements are varied, over the range of analysis desired. The lines of the test element and the internal standard are read and the log ratios of the various concentrations are determined.

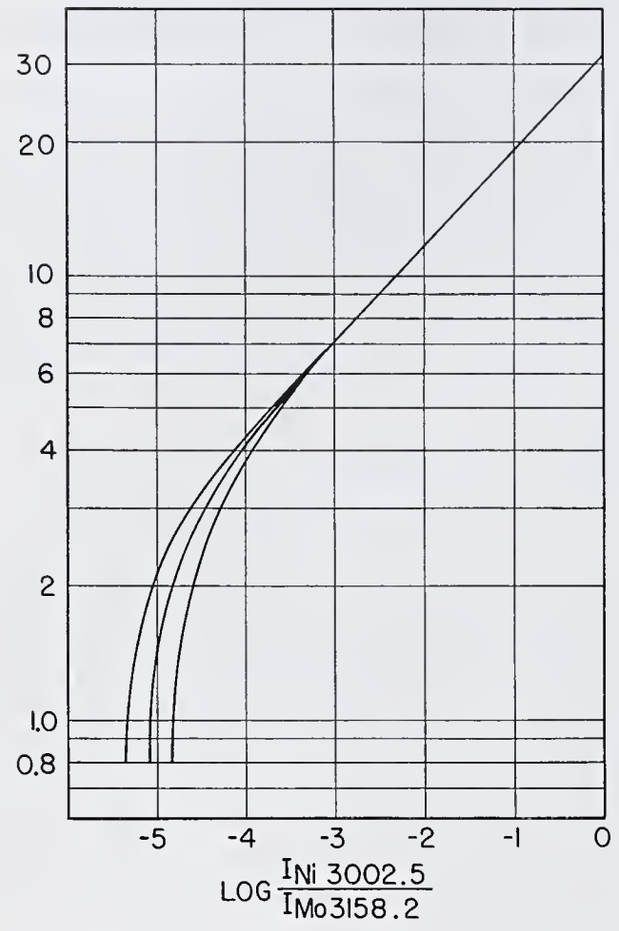

FIGURE 2

The test element is added to a solution of the purest product obtainable to keep the residual amount of the element to a minimum. In many cases this minimum amount is in the range of analysis desired. Therefore, it is desirable to determine this residual amount as accurately as possible. This is not always possible by chemical methods because of the small amount of the element present.

The procedure used for determining the residual, using aluminum as the impurity in a solution of 25 per cent of sodium hydroxide as an example, is as follows:

The solutions, containing varying amounts of aluminum (beginning with a solution containing no added aluminum) and the constant amount of molybdenum, are prepared, coated on the electrodes, and burned, and the spectrograms are made. The lines and the plate-calibrating pattern are read on the microphotometer and the relative intensities of the lines are determined. Then the log differences of the relative intensities of the aluminum line and the molybdenum line are determined.

A value is assumed for the residual aluminum in the first solution and this is added with the additional amounts in the other solutions. These concentrations are plotted against the respective log intensity ratios, using semilog paper. If the assumed quantity of aluminum is higher than actually present, the curve will bend upward (Figure 3, A). If the assumed value is low, the curve will bend down, *B*. When the actual amount is assumed, the curve will become a straight line, *C*.

By overcorrecting and undercorrecting the calibrating curve, and approaching the corrected value with a series of curves on both sides (Figure 3, *E*), the amount of the impurity in the sample used for calibration can be determined to within 20 per cent of the actual amount present.

This method of correction is shown also in a case where the lower end of the curve bends down, owing to the effect of the background (Figure 4). Curve *A* is overcorrected, *B* is undercorrected, while in *C* the correct value is assumed.

This method of preparing working curves is based on the linear relation obtained when plotting the log ratio of the in-

TABLE I. PRECISION AND RANGE OF ANALYSES

	Spectral Lines Impurity	Mo	Range of Analysis	Deviation from Mean
	Å.	Å.	%	%
		25% Sodium Hydroxide		
Si	2881.6	3158.2	0.0005 –0.0050	3.2
Si	2987.7	3158.2	0.0190 –0.0700	4.2
Al	3092.7	3158.2	0.00015–0.0010	2.0
Al	3082.2	3158.2	0.0002 –0.0015	2.6
Al	2660.4	3158.2	0.0010 –0.0040	4.0
Ca	3968.5	3903.0	0.0001 –0.0015	4.5
Mg	2802.7	2816.2	0.0004 –0.0010	4.3
Fe	2483.3	2649.5	0.00007–0.0005	5.0
Mn	2794.8	2649.5	0.00002–0.00005	3.0
Mn	2794.8	3158.2	0.00005–0.0001	1.8
Cu	3247.6	[a]	0.00004–0.0002	2.5
Ni	3002.5	[a]	0.00008–0.0006	2.6
Ni	3050.8	[a]	0.00008–0.0006	3.2
Sr	4077.7	3903.0	0.0001 –0.0020	7.8
Pb	2833.1	3158.2	0.00002–0.0003	8.0
		10% Sodium Hydroxide		
Si	2881.6	3158.2	0.0002 –0.0030	2.2
Al	3092.7	3158.2	0.00005–0.0006	4.6
Al	3082.2	3158.2	0.0001 –0.0015	3.9
Ca	3006.9	3158.2	0.0010 –0.0110	6.8
Mg	2779.9	3158.2	0.0001 –0.0036	7.4
Mg	2783.0	3158.2	0.0009 –0.0050	6.9
Fe	2483.3	3158.2	0.00005–0.0002	7.3
Mn	2794.8	3158.2	0.00002–0.0002	3.0
		10% Sodium Carbonate		
Si	2881.6	3208.9	0.00006–0.0005	5.9
Ca	3006.9	3208.9	0.0004 –0.0050	7.6
Al	3092.7	3208.9	0.00001–0.0003	5.2
Mg	2779.9	3208.9	0.0002 –0.0020	5.6
Mg	2783.0	3208.9	0.0002 –0.0020	5.5
Fe	3020.7	3208.9	0.00004–0.0004	6.6
		20% Sodium Chloride		
Mg	2802.7	2816.2	0.00006–0.0006	3.1
Ca	3968.5	3903.0	0.0001 –0.0007	4.8

[a] Background next to line.

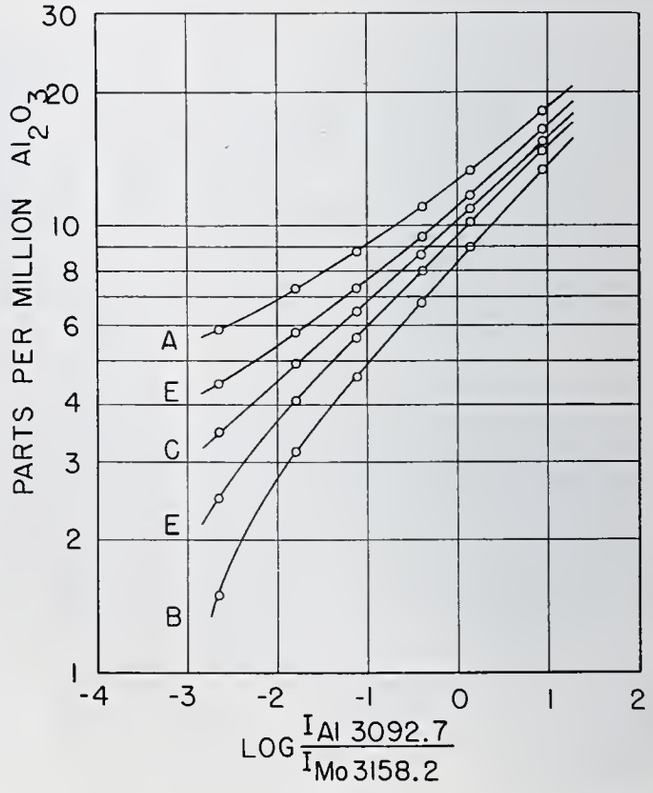

FIGURE 3

TABLE II. ANALYSIS OF SODIUM HYDROXIDE

Analysis No.	Iron	Deviation[a]	Manganese	Deviation	Magnesium	Deviation	Silicon	Deviation	Aluminum	Deviation
	P. p. m.	%	P. p. m.	%	P. p. m.	%	P. p. m	%	P. p. m.	%
1	2.0	4.2	0.36	2.2	3.7	8.0	19	5.6	4.9	3.2
2	1.9	1.0	0.39	6.0	4.2	12.6	18	0.0	5.2	2.8
3	1.85	3.6	0.36	2.2	3.4	8.8	18	0.0	5.1	2.0
4	1.9	1.0	0.36	2.2	3.3	11.5	17	5.6	4.9	3.2
5	2.0	4.2	0.36	2.2	3.5	6.2	18	0.0	5.2	2.8
6	1.95	1.6	0.37	0.5	4.0	7.2	18	0.0	5.0	1.2
7	1.9	1.0	0.37	0.5	3.3	11.5	18	0.0	5.1	0.8
8	2.15	11.4	0.38	3.2	3.9	4.6	19	5.6	5.2	2.8
9	1.95	1.6	0.36	2.2	3.6	3.5	18	0.0	5.1	0.8
10	1.7	11.4	0.36	2.2	3.5	6.2	17	5.6	5.1	0.8
11	1.85	3.6	0.37	0.5	3.9	4.6	18	0.0	5.0	1.2
12	1.9	1.0	0.37	0.5	3.8	1.9	18	0.0	5.0	1.2
13	2.0	4.2	0.36	2.2	4.0	7.0	18	0.0	4.9	3.2
14	1.95	1.6	0.37	0.5	3.9	4.6	18	0.0	5.2	2.8
15	1.7	11.4	0.37	0.5	3.9	4.6	18	0.0	5.0	1.2
16	1.95	1.6	0.37	0.5	3.8	1.9	18	0.0	5.0	1.2
	Av. 1.92	4.0	0.368	1.8	3.73	6.6	18	1.4	5.06	2.0

[a] % deviation from average.

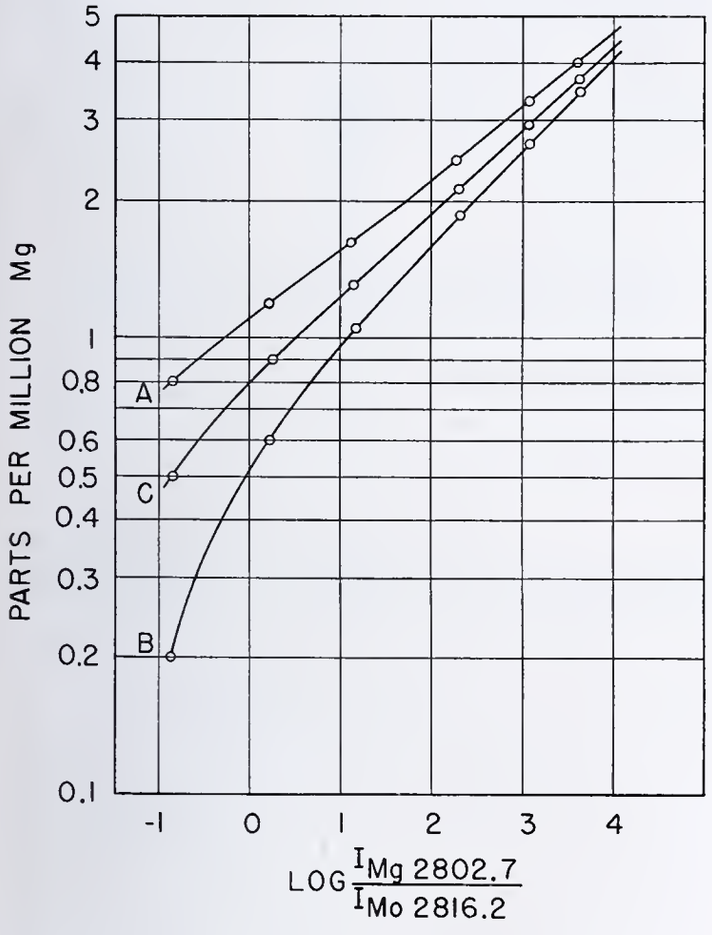

FIGURE 4

tensities of the two lines (impurity and control) against the log of the concentration.

This relationship may be affected by extraneous factors, causing it no longer to hold true. Therefore, it has been customary with this laboratory, when making a new calibration, to calibrate two lines of the impurity so that one line may be checked against the other. However, the only interfering factor encountered in the low concentration range described here has been the effect of the background on the very faint impurity line.

The effect of the background can be overcome to an appreciable extent, by working for the following conditions:

Choose impurity and control lines near each other, so that there is little difference in the background around the lines.

Adjust the concentration of the internal standard, so that the intensity of the control line is near the intensity of the impurity line.

Analysis of Sodium Carbonate and Bicarbonate

The sodium carbonate is dissolved in distilled water to give a 10 per cent solution and 25 p. p. m. of molybdenum standard are added. The electrodes are coated, dried, and burned as in a caustic analysis.

Because of the wide range of analysis covered by various grades of ash, it is necessary to use several lines for some of the elements. The lines used and the range of analysis are shown in Table I.

Sodium bicarbonate is first calcined to sodium carbonate, and then the sodium carbonate procedure and curves are used. If the sodium bicarbonate is burned at 200° C. for 1 hour, there is no loss nor gain of impurities.

Analysis of Sodium Chloride

The sodium chloride comes into the laboratory as a saturated solution (25 to 26 per cent sodium chloride), which is diluted to 20 per cent sodium chloride with 25 p. p. m. of molybdenum as internal standard.

If this 20 per cent salt solution is coated on an electrode and burned, the arc will burn through one spot on the electrode, and after the salt in this spot is gone, the arc burns on bare carbon only. This tends to make very erratic analytical results. It can be avoided by adding sufficient redistilled nitric acid to make the final solution approximately 7 per cent nitric acid. The arc will then wander over the entire face of the electrode. Using this method, very reproducible results are obtained.

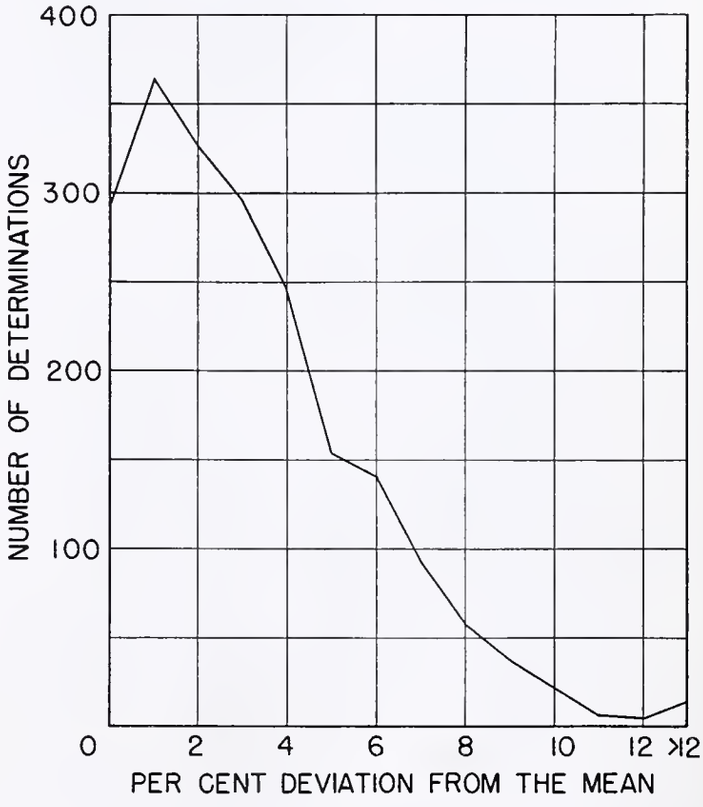

FIGURE 5

Accuracy and Precision

It is possible to determine the amount of impurity in the sample used for calibration to within 20 per cent of the amount present. Therefore, if the sample to be analyzed is as pure as the sample from which the calibration was made, the analysis may be ±20 per cent from the actual amount. This error decreases on analyses made farther up on the calibration curve. If there is again as much impurity in the sample analyzed as in the sample from which the calibration was made, the possible error will be half, or 10 per cent.

The precision of analysis, the range of analysis, and the spectral lines used are shown in Table I.

The precision (per cent deviation from the mean) was calculated from control analyses made in duplicate over a period of 3 months. During this time, 3600 determinations were made.

The distribution of the deviations from the mean for 25 per cent sodium hydroxide is given in Figure 5. Any duplicate determinations having deviations greater than 10 per cent are not accepted and the analysis is repeated. Less than 2 per cent of the analyses lie in this range.

The precision of analysis was also calculated for a sample of 25 per cent sodium hydroxide analyzed sixteen times over a period of 24 days (Table II). No two of these analyses were made on the same photographic plate. Calcium and copper were not determined because a separate exposure is necessary. Nickel, lead, and strontium were below the limit of detection.

Acknowledgment

The author wishes to thank O. S. Duffendack and R. A. Wolfe for suggestions and assistance rendered in the installation and operation of the laboratory and the Michigan Alkali Company for permission to publish the analytical data included in this paper.

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Presented at the Second Midwest Conference on Spectrographic Analysis, University of Chicago, March 29, 1941.

Rapid Determination of the Specific Gravity of Plastic Materials

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The specific gravity of a plastic material is indicated directly by a machine as a result of the controlled compression of a known weight of sample. The specific gravity of unvulcanized reclaimed rubber or rubber compounds may be determined in approximately one minute.

THE usual method of measuring the specific gravity of reclaimed rubber requires a number of time-consuming operations. The reclaimed rubber, sulfur, and accelerator must be weighed and mixed on a mill, and the mixture vulcanized in a press. The specific gravity is then determined on the vulcanized sample by any method in which the sample is weighed in air and in water, or by testing for buoyancy in a series of salt solutions of known specific gravity.

The purpose of this paper is to describe a mechanical device for measuring very rapidly and accurately the specific gravity of reclaimed rubber, rubber compounds, or other materials which are plastic at room temperature.

Description

The specific gravity is determined in this machine by measuring mechanically the volume of a weighed sample of plastic material when compressed under a definite pressure, the result being indicated on a calibrated dial.

Although the machine actually measures density instead of specific gravity, the results correspond to the specific gravity as measured by other methods. Specific gravity at 20° C. equals 1.002 times density, and compensation is made for this small difference by compressing the sample to slightly greater than its normal cured density.

The principle of the machine is illustrated in Figure 1. The weighed sample is compressed in the hardened steel measuring cylinder, *A*, which has a diameter of 5.08 cm. (2 inches). The cylinder is covered on the upper end by the quick-opening cap, *B*. Pressure is applied to the sample by the bronze piston, *C*, which is moved up and down in the measuring cylinder by the double-acting hydraulic piston and cylinder assembly, *D* and *E*. The position of piston *C* is indicated on the 15.2-cm. (6-inch) dial, *F*, by a pointer rotated by a rack and pinion mechanism. The motion of the piston is transmitted to the dial by the push rod, *G*, which is behind the piston rod and connected to it at *H*. Piston *D* is moved by means of hydraulic pressure controlled by the quick-acting four-way valve, *I*. A constant hydraulic pressure of 28.1 kg. per sq. cm. (400 pounds per sq. inch), indicated on gage *N*, is maintained by diaphragm valve *J*, controller *K*, and air cushion *L* to give a pressure on the sample of 112.5 kg. per sq. cm. (1600 pounds per sq. inch). The compressive force on the sample is 2280 kg. (5020 pounds) with no correction for friction loss.

Since density is defined as mass per unit volume and since the weight of the sample and the diameter of the measuring cylinder are constant, the length of the space occupied by the compressed sample varies inversely with its density. This length is indicated on dial *F*, which is calibrated to read specific gravity directly in the range of 0.960 to 1.850 in intervals of 0.010. The divisions range in size from 8.90 to 2.54 mm. (0.35 to 0.10 inch), and the third decimal place is easily estimated over the entire scale.

Convenience and speed of operation were dominant factors in the design of the machine. The indicating dial is at eye level for easy and accurate reading. The compression chamber and operating valve are placed at a convenient working height. The cap for the cylinder is suspended by a spring on a swinging arm fitted with positioning stops so that the cap may be swung into place and out of the way rapidly, as shown in Figures 2 and 3. An interrupted thread allows locking or releasing of the cap with

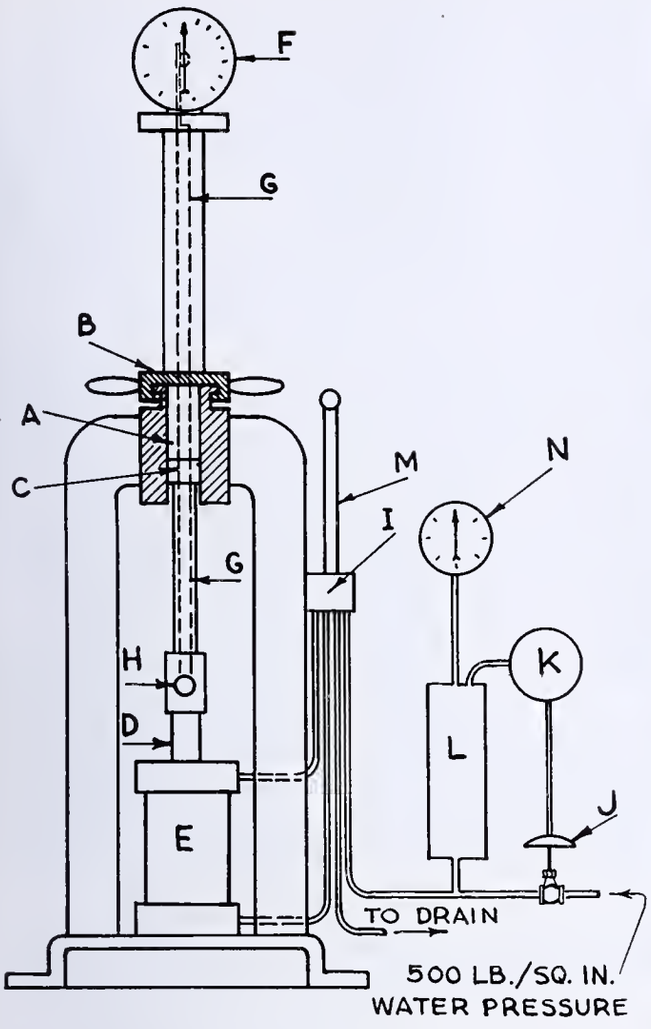

FIGURE 1. DIAGRAM OF MACHINE

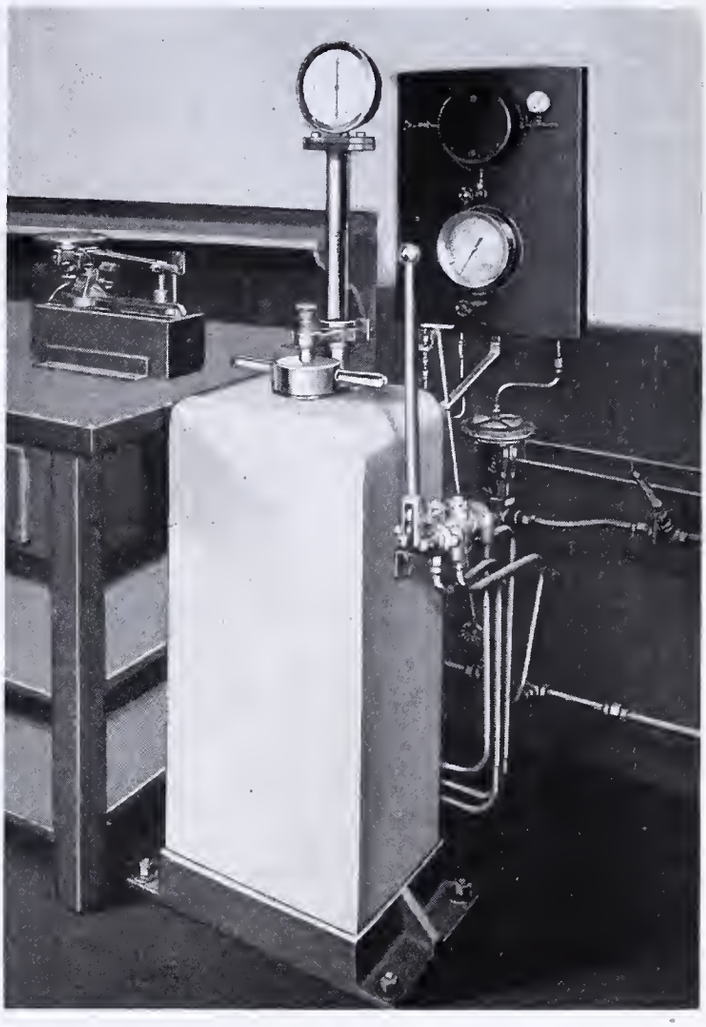

FIGURE 2

a one-eighth turn of the handles. The floor space required is small, since the base of the machine is 58 × 33 cm. (23 × 13 inches).

Operation

In operation, a 100.0-gram sample of reclaimed rubber or other material to be tested is cut into pieces which will enter the 5.08-cm. (2-inch) opening of the cylinder and is then placed in the cylinder, after which the cap is closed. The lever *M*, of the operating valve is pulled forward, causing piston *C* to compress the sample, whereupon the specific gravity is read immediately from the dial. The lever is then pushed back, which releases the pressure momentarily, thereby allowing the cap to be removed. By bringing the lever forward again, the sample is ejected by the piston. The piston is lowered and the machine is ready for the next determination.

The complete operation of weighing a sample and determining the specific gravity is accomplished in approximately one minute. In the case of reclaimed rubber, the sample is frequently hot and must be cooled to room temperature before testing, and this increases the elapsed time of a determination to 10 minutes. With the older methods of measuring the specific gravity of reclaimed rubber, 5 to 10 minutes of working time were required per determination, and the elapsed time was about 30 minutes.

Factors Influencing Accuracy

Several factors influence the accuracy of the machine. The sample must be weighed to 100.00 ± 0.05 grams to eliminate variations from this source. For a specific gravity

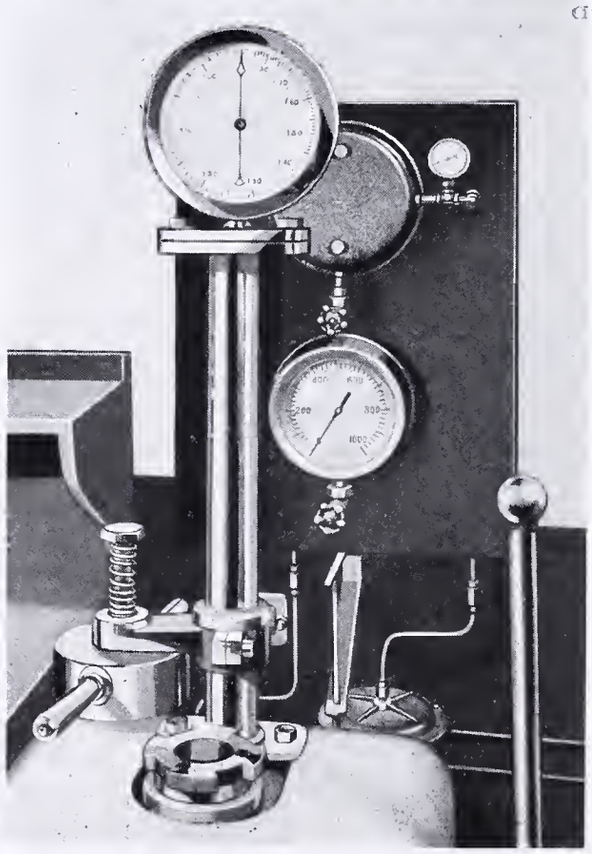

FIGURE 3

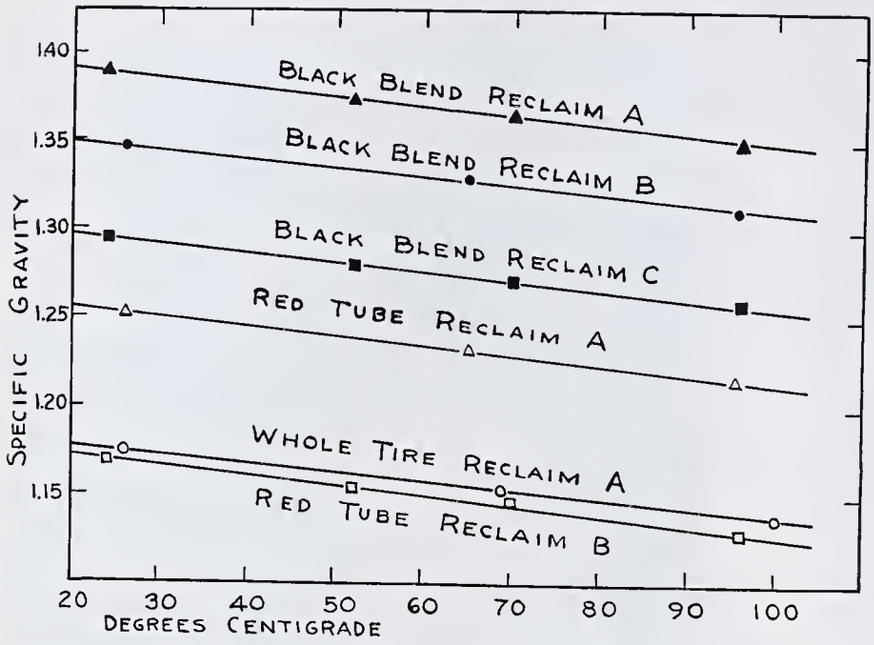

FIGURE 4. EFFECT OF TEMPERATURE ON SPECIFIC GRAVITY OF RECLAIMED RUBBER

of 1.000 the error from this variable is ±0.0005 and for a sample with a specific gravity of 2.000 the error amounts to ±0.001. Since changes smaller than 0.001 cannot be seen on the dial, this error is small. The sample is usually weighed to ±0.1 gram for factory control work in order to save time. The weight of the sample may be changed from 100.00 grams to test materials with a specific gravity outside the range of the dial (0.960 to 1.850) if proper corrections are made. If, for example, a sample of 80.00 grams is used, the indicated reading is multiplied by 0.8.

Temperature has an appreciable effect on the specific gravity of reclaimed rubber, as shown in Figure 4. A rise of 1° C. lowers the specific gravity of reclaimed rubber about 0.0005. The temperature coefficient of volume expansion (increase in unit volume per degree centigrade) as calculated from these curves ranges from 37×10^{-5} to 51×10^{-5} for various types of reclaimed rubber at 25° C. The temperature coefficient for purified crude rubber has been given (*1*) as 67×10^{-5} at 25° C. Room temperature (24° to 30° C.) is sufficiently constant to maintain satisfactory accuracy for factory control work. The samples to be tested are placed on wire trays across which air is circulated by a fan, and in 10 minutes are cooled to room temperature.

Variation in the pressure exerted on the reclaimed rubber causes variation in the specific gravity, as shown in Figure 5. The coefficient of compressibility of whole-tire reclaim is shown within the limits of these curves to be 52×10^{-6} (contraction in unit volume per atmosphere) at 25° C. Scott (*2*) found the coefficient for rubber vulcanized with 3 per cent sulfur to be 48×10^{-6} in the same pressure range. A pressure of 112.5 kg. per sq. cm. (1600 pounds per sq. inch) was selected by experiment as that which compressed the sample to a density corresponding to the specific gravity results obtained with other methods. The air in the compression cylinder is pressed out at the cap through a clearance of 0.025 mm. (0.001 inch), which is too small to pass any rubber. A small amount of air is probably entrapped, but it is compressed to an insignificant volume, and no variations in specific gravity can be shown from this source. The hydraulic pressure which operates the machine is controlled to 28.1 ± 1.4 kg. per sq. cm. (400 ± 20 pounds per sq. inch) by the system described above. Since a variation of 7.0 kg. per sq. cm. (100 pounds per sq. inch) is necessary to cause a measurable variation in the specific gravity, errors from this source are eliminated.

When very plastic reclaim is tested, a film of the sample is left on the walls of the compression chamber. The error resulting from the volume of this film being added to the next sample is negligible because its weight varies from 0.00 to 0.05 gram. This does not have a measurable effect on the result, as shown above in the discussion on weight of the sample.

The maximum error of the machine as operated for factory control tests is shown in Table I to be 0.32 per cent for a sample with a specific gravity of 1.000. The maximum error for a sample with a specific gravity of 2.000 is 0.0042 or 0.21 per cent. When necessary for exact work, all the variables may be controlled to reduce the maximum error to the point where the third decimal place of the result is not affected.

The errors in the usual methods of specific gravity measurement are much larger than those shown above. Microscopic porosity of vulcanized samples of reclaimed rubber can cause variations of at least 0.010 in the specific gravity, a factor which alone is much larger than the total experimental error of the machine method. Other variations may result from

TABLE I. EFFECT OF VARIABLES ON ACCURACY OF SPECIFIC GRAVITY

	Factory Control Tests		Exact Tests	
Variable	Maximum error	Effect on specific gravity	Maximum error	Effect on specific gravity
Weight of sample	±0.1 gram	±0.0010[a]	±0.001 gram	±0.0000
Temperature of sample	±3° C.	±0.0015	±0.5° C.	±0.0003
Hydraulic pressure	±1.4 kg. per sq. cm.	±0.0002	±0.7 kg. per sq. cm.	±0.0001
Residue in cylinder	+0.05 gram	−0.0005	±0.000 gram	±0.0000
Total maximum error	.....	+0.0027 −0.0032	.....	±0.0004

[a] For a sample with a specific gravity of 1.000.

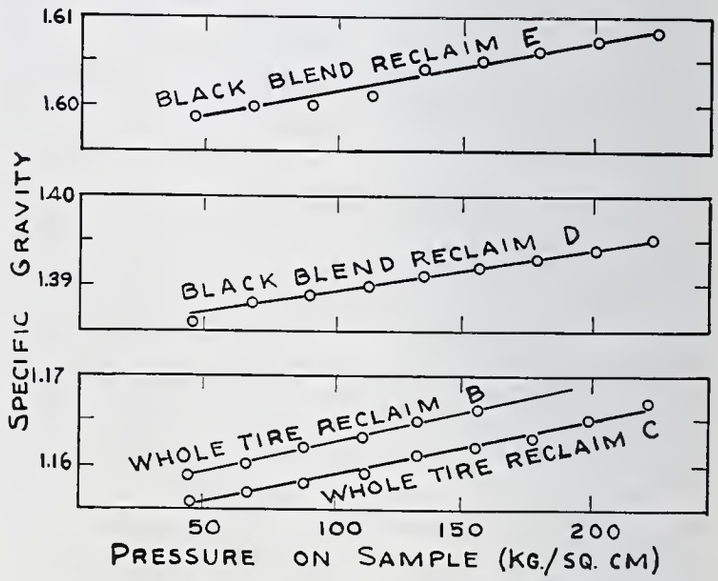

FIGURE 5. EFFECT OF PRESSURE ON SPECIFIC GRAVITY OF RECLAIMED RUBBER

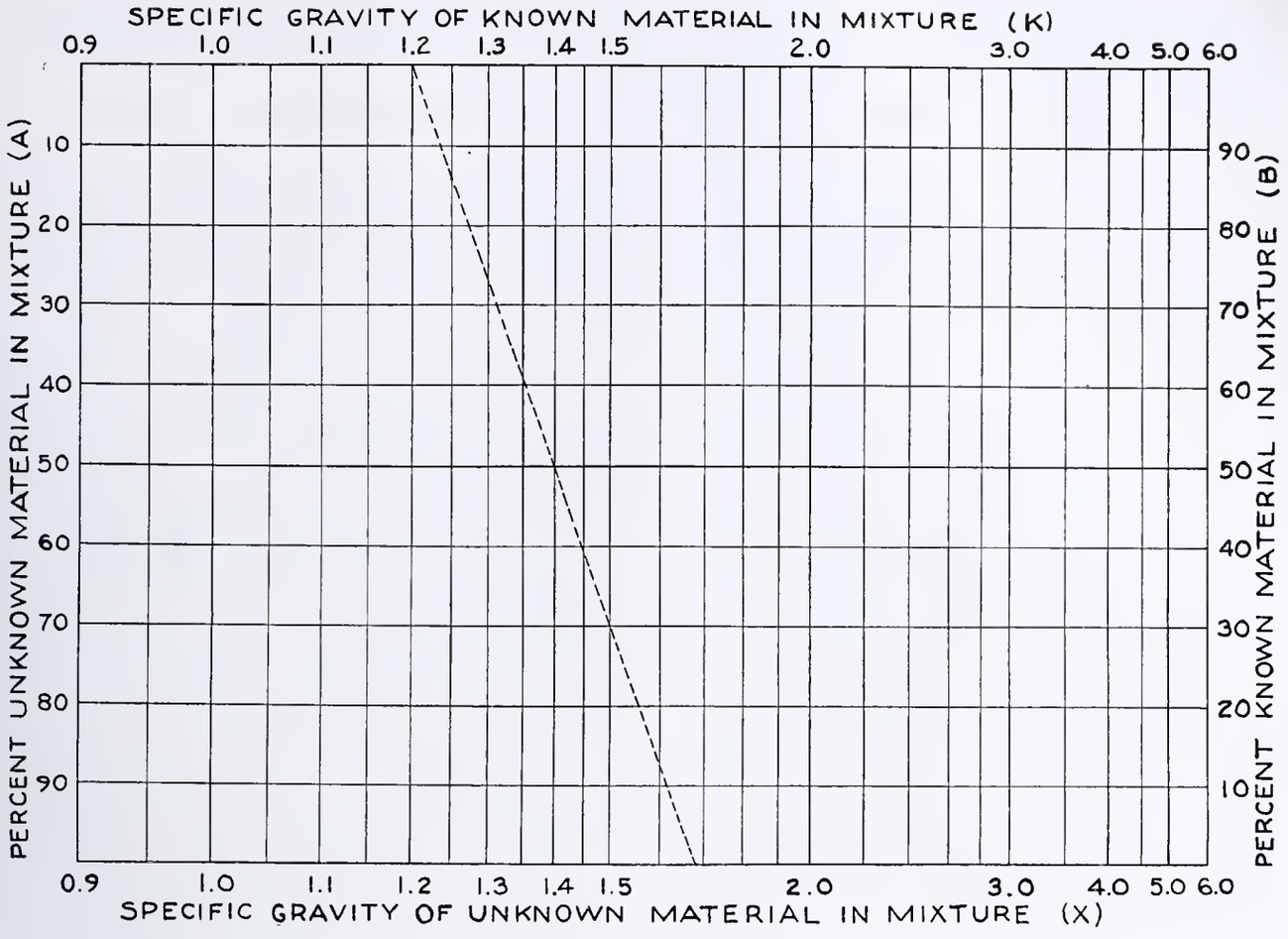
SPECIFIC GRAVITY OF KNOWN MATERIAL IN MIXTURE (K)
0.9 1.0 1.1 1.2 1.3 1.4 1.5 2.0 3.0 4.0 5.0 6.0
PERCENT UNKNOWN MATERIAL IN MIXTURE (A)
10 20 30 40 50 60 70 80 90
PERCENT KNOWN MATERIAL IN MIXTURE (B)
90 80 70 60 50 40 30 20 10
0.9 1.0 1.1 1.2 1.3 1.4 1.5 2.0 3.0 4.0 5.0 6.0
SPECIFIC GRAVITY OF UNKNOWN MATERIAL IN MIXTURE (X)

FIGURE 6. SPECIFIC GRAVITY OF A TWO-COMPONENT MIXTURE

$$X = \frac{A}{\frac{100}{M} - \frac{B}{K}}$$

M = specific gravity of mixture (on horizontal lines)

Directions. Mark specific gravity of known component on top scale and specific gravity of mixture on horizontal line which corresponds to percentage composition of mixture. Connect these points with a straight line and read specific gravity of unknown on bottom scale.

Example (dotted line). Specific gravity of known = 1.20. Specific gravity of 50 per cent mixture with unknown = 1.40. Specific gravity of unknown = 1.68.

errors when weighing and mixing the sulfur and accelerator with the sample, from varying vulcanization periods and temperatures, and from incomplete wetting of the vulcanized sample when weighing in water. The method which makes use of a series of salt solutions of known specific gravity is the most dependable and rapid of the older methods for checking the specific gravity of vulcanized rubber samples, but the accuracy is limited to the interval between consecutive solutions, which for practical purposes is usually 0.010. Hence, in these methods a total error of 0.020 is not uncommon.

Applications

This machine can be used to measure the specific gravity of unvulcanized natural or synthetic rubber compounds or devulcanized scrap rubber in process of manufacture without additional treatment. Its value is most apparent when testing materials which ordinarily must be mixed with vulcanizing ingredients to obtain a cured sample for the usual specific gravity methods.

The specific gravity of nonplastic materials such as rubber compounding materials or ground scrap rubber may be determined if mixed with a plastic material such as crude or reclaimed rubber. The plastic material must be present to flow under pressure and fill the voids between the nonplastic particles. It is usually convenient to mix equal parts of the nonplastic material and reclaimed rubber of a known specific gravity on a mill and to test the mixture in the specific gravity machine. The unknown component in such a mixture may be found by using the formula or alignment chart shown in Figure 6.

Summary

A machine for the determination of the specific gravity of reclaimed rubber or other plastic materials has been described. The determination is made on an unvulcanized sample which is compressed to 112.5 kg. per sq. cm. (1600 pounds per sq. inch) in the case of reclaimed rubber and rubber compounds, and the specific gravity is indicated on a dial. The time required for a determination is approximately one minute. The weight and temperature of the sample and the pressure applied to it affect the result, but with proper control of these variables the specific gravity is reproducible to the third decimal place. This is much more accurate than other specific gravity methods for rubber compounds.

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PRESENTED before the Division of Rubber Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Mo.

Methods of Light Hydrocarbon Analysis

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Various methods for the analysis of mixtures of light hydrocarbons from methane through isopentane have been studied and compared as to their accuracy and suitability for routine operations. The methods studied have included the automatically controlled Podbielniak (Model L) column, the Ward method utilizing low-pressure fractional distillation and condensation, a dew-pressure method for *n*-butane–isobutane mixtures, the determination of olefins by absorption in various reagents and by catalytic hydrogenation, and the determination of isobutene by absorption in various reagents and by its reaction with anhydrous hydrogen chloride. Synthetically prepared mixtures have been used for the fundamental investigations. Various methods of sampling and handling liquefied light hydrocarbon mixtures have been studied and compared.

IN RECENT years, the various light hydrocarbons have assumed an increasingly important role as raw materials and there is every indication that this trend will continue. The application of conversion and polymerization processes for the production of gasoline stocks has been a prime factor in the demand for certain light hydrocarbon stocks of definite composition. Concurrently with these developments, there has been an increasing interest in the reproducibility and accuracy of methods of analysis for these light paraffins and olefins, especially the group of four-carbon-atom hydrocarbons.

The present work has included studies of a number of the more widely accepted methods of analysis. Where results indicated it to be desirable, the methods were modified. In many cases, the methods were subjected to a theoretical analysis of the maximum accuracy to be expected. Consideration was given to accuracy, reproducibility, simplicity, speed, and economy of materials. Synthetically prepared mixtures were used for the fundamental investigations, and studies were made of methods of sampling and handling the mixtures prior to analysis.

The types of apparatus studied for the fractional analysis of light hydrocarbon mixtures were the automatically controlled Podbielniak column, the modified California Natural Gasoline Association's (McMillan) column (*6*), and a modification of the Ward apparatus (*15*) for the fractionation of light hydrocarbons at reduced temperatures and pressures. In conjunction with the study of these apparatus, a dew-pressure apparatus for the determination of isobutane and *n*-butane in their two-component mixtures was developed. Included in the study of methods for unsaturate analysis were the determination of total unsaturates in gaseous samples by absorption in various reagents and by the McMillan catalytic hydrogenation method (*7*), and the determination of isobutene in gaseous samples by absorption in various reagents and by the McMillan anhydrous hydrogen chloride method (*5*).

Purification of Gases and Blending of Synthetic Mixtures

In order to obtain exact and comparable data as to the relative and absolute accuracies of the methods of analysis that were studied during this investigation, a large number of analyses were made using synthetic mixtures of known composition, accurately blended from purified gases. In addition to the analyses made with synthetic samples, numerous routine plant samples were analyzed to determine the applicability of the apparatus to routine testing work.

In general, the procedure employed for the purification of the saturated hydrocarbons used in preparing synthetic blends consisted of scrubbing the gas (to remove such impurities as unsaturates, carbon dioxide, sulfur dioxide, etc.) and fractionating the scrubbed gas in a Podbielniak column to remove higher- and lower-boiling impurities. The apparatus employed for the purification of these gases consisted of (*a*) a series of six glass scrubbers, three of which were packed with glass beads and contained sulfuric acid in concentrations ranging from 79 to 20 per cent fuming and the remaining three of which contained 43 per cent potassium hydroxide solution, Ascarite, and calcium chloride pellets; (*b*) a Podbielniak column, to which the scrubbed gases were charged; (*c*) a condensation bulb and 11.4-liter (3-gallon) bottle for collecting pure and impure fractions, respectively, from the Podbielniak distillation; and (*d*) suitable connections to the storage and blending apparatus and to the vacuum line.

The ethane, propane, isobutane, and *n*-butane used in this work were purified from commercial gases according to the procedures described by McMillan (*6*). Isopentane was purified by a series of prolonged distillations in which the front and tail ends from each distillation were discarded. Isobutene was prepared by contacting *tert*-butyl alcohol and oxalic acid in equal parts by weight in a suitable apparatus equipped with a stirring device and reflux condenser, the evolved gases being repeatedly scrubbed with caustic and condensed before being tested for purity by absorption in 94 per cent sulfuric acid. The 2-butene was prepared by contacting *sec*-butyl alcohol and phosphoric acid, the apparatus and procedure for treating the evolved gases being the same as for isobutene. Constancy of boiling point (within 0.1° C.) after repeated fractionations in which the front and tail ends from each distillation were discarded was the criterion employed for determining the purity of saturated hydrocarbons; in the case of isobutane and *n*-butane, this test was usually supplemented by dew-pressure determinations.

After purification, the gases were transferred to the storage and blending system. This system consisted essentially of four banks of five 11.4-liter (3-gallon) bottles each, all the bottles in each bank being connected to a common manifold, which was in turn connected to the purification system and to the vacuum line. Each bank of bottles was connected to a condensation bulb, a Y-type manometer, and a calibrated receiver (immersed in a water bath) of approximately 2000-ml. capacity. Each receiver was connected through a second manifold to the vacuum line and to the analytical equipment. A Toepler pump and condensation bulb, together with manometers and calibrated receivers, were also connected to this manifold. All apparatus used in the blending and transferring of synthetic mixtures was constructed of fused glass.

When preparing a synthetic mixture, the desired portion of gas in each bank of bottles was transferred to its respective receiver by condensation and revaporization in the condensation bulb. To ensure the removal of any air that may have leaked through stopcocks into the storage bottles during prolonged storage periods, the condensation bulbs were always opened to vacuum for approximately 15 minutes after the gas had been condensed with liquid nitrogen. The molar volume of each gas was determined by manometer readings in the respective receivers, all of which were immersed in a common water bath to eliminate errors caused by possible temperature differences. Inasmuch as all receivers were calibrated with respect to volume, a simple calcu-

Table I. Automatic Podbielniak Analyses

	Refinery Sample Run 1	Refinery Sample Run 2	Refinery Sample Average
	Mole per cent		
Propane	2.3 ± 0.2	1.9 ± 0.2	2.1 ± 0.2
Isobutane	83.1 ± 0.2	83.0 ± 0.2	83.0 ± 0.2
n-Butane	14.6 ± 0.2	15.1 ± 0.2	14.9 ± 0.2

	Synthetic Mixture 1	Synthetic Mixture 2	Synthetic Mixture 3	Analysis of Synthetic Mixture 1	Analysis of Synthetic Mixture 2	Analysis of Synthetic Mixture 3
	Mole per cent			*Mole per cent*		
Propane	2.6 ± 0.2	....	3.1 ± 0.2	2.7 ± 0.2	....	3.2 ± 0.2
Isobutane	80.8 ± 0.2	....	95.8 ± 0.2	80.6 ± 0.2	....	95.6 ± 0.2
n-Butane	16.6 ± 0.2	4.8 ± 0.2	....	16.7 ± 0.2	4.7 ± 0.2	....
Butenes	....	....	....	....	....	....
Isopentane	....	8.4 ± 0.2	1.1 ± 0.2	....	8.4 ± 0.2	1.2 ± 0.2
n-Pentane	....	6.5 ± 0.2	....	....	6.4 ± 0.2	....
Hexanes	....	19.2 ± 0.2	....	....	19.0 ± 0.2	....
Heptanes and heavier	....	61.1 ± 0.2	....	....	61.5 ± 0.2	....

lation, based on the difference in pressure in the receivers before and after removal of the gas, evaluated the quantity of each gas in the blend. All gases used in the blend were, after measurement, condensed in a common condensation bulb, from which the blended mixture was revaporized and transferred by means of the Toepler pump to any part of the apparatus desired. Before introducing gases into the blending system, the pressure in the system was reduced to 0.001 mm. of mercury or less (as determined by a McLeod gage) with the aid of a mercury diffusion pump.

To compensate for errors in blending introduced by deviations from the ideal gas laws, the measured volume of each gas has been corrected to its volume as an ideal gas, using the compressibility data of Jessen and Lightfoot (*3*) and Roper (*10*). These corrections usually amounted to less than 0.4 mole per cent of any component based on the total sample. Assuming manometric readings to be accurate to within ±0.5 mm. of mercury, the over-all probable uncertainty in blending has been computed to be between ±0.1 and ±0.2 mole per cent on each component based on the total sample. The probable uncertainties in composition incurred when measuring gas volumes or pressures in blending and in analyzing by the various methods have been included in the data tables.

Automatically Controlled Microfractionation Columns

Criticism has been directed in recent years toward standard microfractionation columns because of their failure to give consistent, reproducible analytical results. In this laboratory, serious discrepancies were sometimes encountered when duplicate samples were analyzed on different columns, or by different operators on the same column. Although it was suspected that these discrepancies were attributable more to variations in the operating technique employed by different operators and minor differences in the construction of the columns themselves than to any fundamental fault of the columns, the lack of definite data as to the absolute accuracy of the columns when operated under different conditions precluded, until recently, the establishment of a standardized operating procedure.

Inasmuch as Podbielniak (*8*, *9*) and McMillan (*6*) have reported thorough studies of the various factors affecting the analytical accuracy of the manually controlled Podbielniak and modified California Natural Gasoline Association's columns, this report will be limited to a discussion of results from the automatically controlled columns only.

Although analyses of synthetic mixtures demonstrated that more accurate results could be obtained from the manually controlled modified C. N. G. A. column than from the manually controlled Model L Podbielniak column, it was found that the modified C. N. G. A. column as developed by McMillan was not adaptable to the type of automatic control currently used in this laboratory for operating Podbielniak columns. Although further modifications of the McMillan column (such as removing the aluminum slugs from the condenser head) resulted in improved operation under automatic control, these modifications necessitated the removal of many of the basic features to which McMillan attributed the improved accuracy obtainable in his column. Except for the improved packing in the McMillan column (consisting of a six-turn-per-inch No. 13 gage brass spiral wire with a No. 23 gage straight wire insert), there was little difference between the automatically controlled McMillan and Podbielniak columns. In view of this, only the automatically controlled Podbielniak column was studied during this investigation; it is assumed that comparable results would be obtained from the modified McMillan column.

Several analyses of typical routine and synthetic light hydrocarbon samples in the automatically controlled Podbielniak Model L column have indicated (Table I) that samples containing no olefins can be analyzed with a maximum error of about ±0.3 per cent on any component based on the total sample, including isobutane and *n*-butane, provided the operating conditions are adapted to the type of sample being analyzed and are carefully controlled throughout the distillation. These results compare with an average maximum error of about ±0.7 per cent reported by McMillan for the manually operated Podbielniak "micro" precision column.

This investigation has indicated also that light hydrocarbon samples containing unsaturates may be separated satisfactorily into groups of the same number of carbon atoms in this column, but that fractionation between unsaturates and saturates containing the same number of carbon atoms is extremely difficult except possibly in the case of ethene-ethane. (No attempt has been made to evaluate, in this investigation, the accuracy of the ethene-ethane separation.) It is feasible, of course, to analyze a sample containing unsaturates by operating a fractionating column in conjunction with auxiliary equipment, analyzing segregated fractions by absorption, hydrogenation, or the method described by Lang (*4*).

In order to achieve consistently accurate results from the Podbielniak column, the operator must adhere to the following standardized procedure, with modifications as noted, according to the nature of the sample.

After charging the sample, the temperatures in the still and head of the column are so balanced that the column is operated under total reflux at slightly reduced pressure (about 100 mm. less than atmospheric) for a period of time depending upon the nature of the sample. From 30 to 60 minutes of refluxing is usually sufficient to bring the column to equilibrium. (If the sample contains large quantities of methane, the distillation may be started without bringing the column under total reflux.) The pressure in the column is then slowly increased to atmospheric, and the distillation is begun with the temperature in the head of the column at the boiling point of the lightest component in the sample.

During the distillation, the temperature in the condenser section of the column, as recorded by the potentiometer, is taken as an indication of the purity of the overhead product and, on plateaus, is maintained as near as possible to the true boiling point of the component being withdrawn. If, on a plateau, the temperature should rise above the boiling point of the component

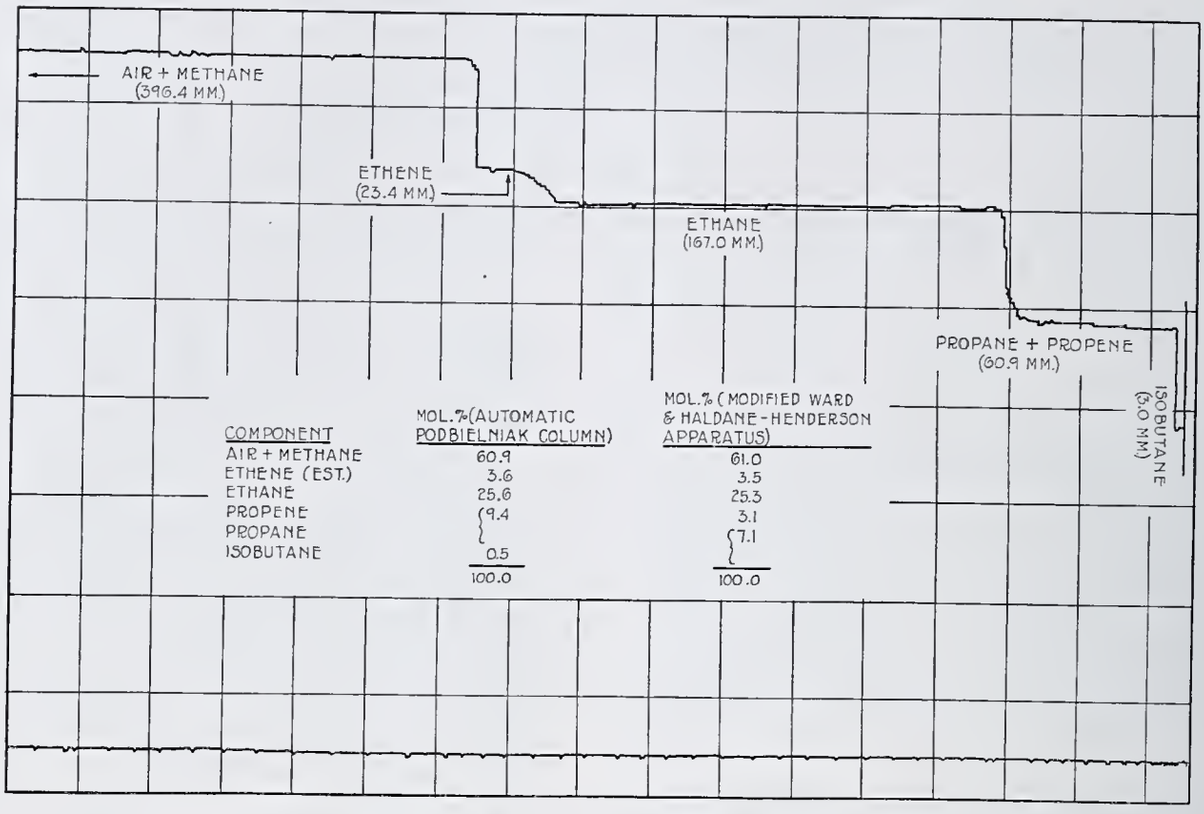

FIGURE 1. AUTOMATIC PODBIELNIAK COLUMN (MODEL L) DISTILLATION CHART

being withdrawn, the take-off should be discontinued immediately and the column operated under total reflux until the temperature returns to its former value. The take-off should then be recommenced at a slower rate and the distillation allowed to proceed. If the temperature should again rise, this procedure should be repeated until a take-off rate which will maintain the plateau temperature at a constant value has been established or until a break is certain.

When a break between components is imminent (as indicated by a decrease of pressure in the column, a "thinning out" of the reflux on the column packing, or both) the column should be operated under total reflux for several minutes while the boiling rate in the still is reduced. By reducing the boiling rate the liquid reflux on the column packing is further thinned out, thus materially reducing the column holdup (while maintaining a high reflux ratio) and increasing the sharpness of the break. After these conditions have been established, the take-off is again begun at a very slow rate and continued until the break is completed. This procedure may be considerably modified for different types of samples. For samples containing large amounts of methane, ethane, or propane, the distillation conditions may be so controlled on plateaus (by controlling the heat input to the still) that the boiling rate in the still is automatically reduced when a break is approached. Under these conditions, the reflux will thin out and the take-off rate will be lowered automatically, thus obviating the necessity for operating the column under total reflux.

In the analysis of gaseous samples, the pressure in the column is reduced to 500 to 600 mm. of mercury and the still is filled with mercury toward the end of the distillation in order to minimize dead space errors within the column. In the analysis of liquid samples, the pressure in the column is reduced to 500 mm. of mercury after the *n*-butane plateau has been reached, and to 250 mm. of mercury after the isopentane plateau has been reached. After each reduction in pressure the column is operated under total reflux for at least 15 minutes to bring it to equilibrium.

When a sample is distilled under the above conditions, the appearance of the finished distillation chart is taken as the best indication of the fractionation, and hence, analytical accuracy, obtained during the analysis. In appearance the distillation curve should have the following characteristics: (*a*) the plateaus should be horizontal, with few fluctuations (such as are caused by excessive pressure variations, flooding in the column, and other undesirable operating conditions), and should show no rise in temperature even when approaching a break; (*b*) breaks between components should be sharp, exhibiting little sloughing off at the plateaus, except in the case of separations between isomers or between saturates and unsaturates containing the same number of carbon atoms. A typical distillation curve, obtained in this laboratory when employing this standardized operating procedure for the analysis of a refinery sample containing unsaturates, is shown in Figure 1. (The percentage of ethene in this distillation was estimated by the equal-area method; however, the accuracy of the ethene-ethane separation has not been evaluated in this laboratory and ethene is usually determined by hydrogenation of the C_2 fraction.)

It is obvious that the accuracy of automatic Podbielniak analyses depends not only upon the skill and experience of the operator but also upon the cleanliness and mechanical conditions of the column and automatic equipment.

Although smooth operation of the automatic Model L type column used in this investigation was considerably enhanced by several minor changes in the automatic control equipment and in methods employed for inducing reflux and controlling the heat input to the still, the necessity for the majority of these modifications has been obviated by the introduction of the new Podbielniak "supercool" column. This column includes certain construction features (such as the extension of the vacuum jacket to cover the still as well as the column, and

TABLE II. CONDITIONS FOR SEPARATION OF COMPONENTS

Separation	Final Pressure	No. 5	Condenser Temperatures No. 4	No. 3	No. 2	No. 1
	Mm. Hg		° *C.*	° *C.*	° *C.*	° *C.*
C_1-C_2	0.03	Liquid nitrogen	−175	−160	−142	−128
C_2-C_3	0.03	Liquid nitrogen	−160	−143	−128	−113
C_3-C_4	0.03	Liquid nitrogen	−136	−122	−107	Room temp.
C_4-C_5	0.03	Liquid nitrogen	−112	−96	Room temp.	Room temp.

Table III. Modified Ward Apparatus Analyses

	Synthetic Mixture				Analysis			
	Run 1	Run 2	Run 3	Run 4	Run 1	Run 2	Run 3	Run 4
	Mole per cent				*Mole per cent*			
Ethane	15.7 ± 0.2	7.6 ± 0.2	70.7 ± 0.2	2.3 ± 0.2	15.4 ± 0.2	7.7 ± 0.2	71.1 ± 0.2	2.4 ± 0.2
Propane	16.2 ± 0.2	41.6 ± 0.2	2.8 ± 0.2	3.4 ± 0.2	16.2 ± 0.2	41.7 ± 0.2	3.1 ± 0.2	3.3 ± 0.2
Butanes	68.1 ± 0.2	42.1 ± 0.2	4.5 ± 0.2	89.9 ± 0.2	68.4 ± 0.2	42.0 ± 0.2	4.3 ± 0.2	90.2 ± 0.2
Isopentane	.....	8.7 ± 0.2	22.0 ± 0.2	4.4 ± 0.2	.....	8.6 ± 0.2	21.5 ± 0.2	4.1 ± 0.2

an improved method for applying liquid nitrogen) which practically eliminate superheating within the still and column.

High-Vacuum Gas Analysis

In general, methods for the fractional analysis of light hydrocarbon mixtures may be divided into two classifications, both of which are dependent upon the relative volatilities (alpha) of the various hydrocarbons in the mixture and to some extent upon rectification. In the type of apparatus developed by Ward (*15*) as a modification of the apparatus originally developed by Shepherd and Porter (*14*) and improved upon by Shepherd (*13*), separation of the components in a mixture is achieved through a series of simultaneous partial distillations and condensations in a series of highly evacuated condenser tubes across which is maintained a temperature gradient (each condenser being maintained at a constant temperature) throughout a given separation. The final separation of each component from the remainder of the mixture is achieved in these apparatus at pressures below 0.1 mm. of mercury absolute, at which pressure the relative volatilities of the various hydrocarbons are several times greater than they are at atmospheric pressure. Thus the fractionating efficiency of this type of apparatus is obtained as a result of these large values of alpha; in a microfractionation column, where the values of alpha are relatively small, effective fractionation is achieved primarily through rectification at low temperatures and at pressures ranging from about 100 mm. of mercury to atmospheric. Consequently, many of the inherent difficulties of a column analysis, such as holdup, maintenance of reflux ratios, etc., are not encountered in the type of apparatus developed by Shepherd and Porter and by Ward. Because of these and other intrinsic advantages, an apparatus similar to that used by Ward was investigated.

As used in this investigation, the modified Ward apparatus (Figure 2), constructed of Pyrex glass and mounted on Transite board, consisted essentially of a condenser train consisting of four condenser tubes for fractionation, plus a fifth condenser for collecting gases distilled from the train; a constant-volume buret with a calibrated capacity of 256.4 cc. for measuring gas quantities; an internal pumping system consisting of a mercury diffusion pump and a Toepler pump (controlled with compressed air);

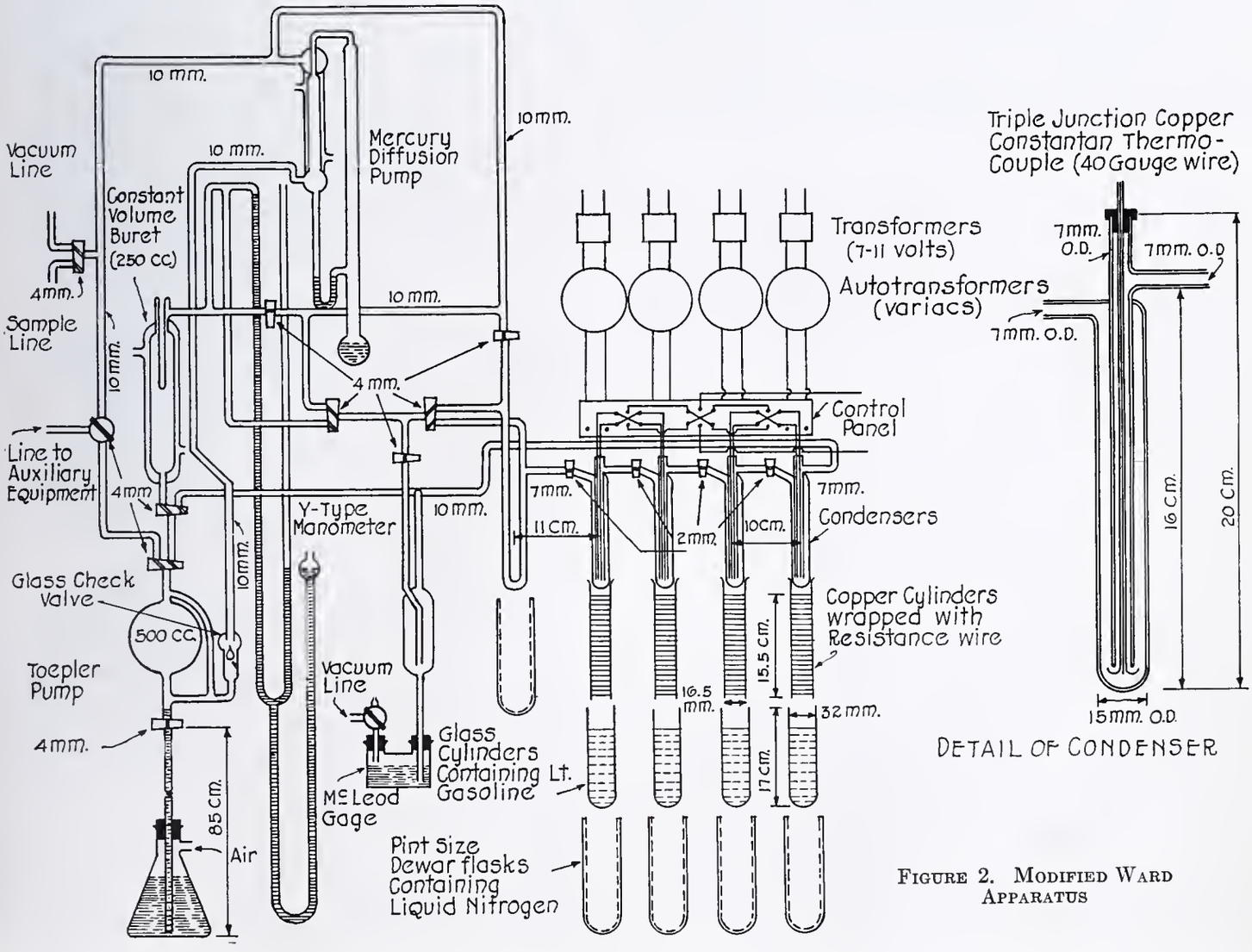

Figure 2. Modified Ward Apparatus

Table IV. Modified Ward Apparatus Analyses

	Straight Sample		Hydrogenated Sample[a]		Average	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
	Mole per cent		*Mole per cent*		*Mole per cent*	
Ethane and ethene	3.6 ± 0.2	.....	3.7 ± 0.3	.....	3.6 ± 0.2	.....
Propane and propene	82.0 ± 0.2	2.1 ± 0.2	82.4 ± 0.3	2.7 ± 0.3	82.2 ± 0.2	2.4 ± 0.2
Butanes and butenes	14.4 ± 0.2	97.5 ± 0.2	13.9 ± 0.3	96.9 ± 0.3	14.2 ± 0.2	97.2 ± 0.2
Isopentane and heavier	.....	0.4 ± 0.2	.....	0.4 ± 0.3	.....	0.4 ± 0.2
Total unsaturate content	20.1 ± 0.2	31.2 ± 0.2	.....	.....	.....	.....

[a] Results calculated on hydrogen-free basis.

and a McLeod gage for measuring the pressure in the condenser trains (controlled by vacuum).

The following system of temperature control was used on each of the four condenser tubes: A copper cylinder wrapped with resistance wire and containing light gasoline was slipped over each condenser and the wire was attached to contact points on the control panel. The contacts for each cylinder were in turn connected to an autotransformer (Variac) and transformer in series, the latter giving a range of 7 to 11 volts and the former giving 0 to 100 per cent control of the transformer voltage. A glass cylinder containing light gasoline and a pint-size Dewar flask containing liquid nitrogen were then slipped concentrically over the copper cylinder. The temperature in the condenser was adjusted and maintained at any desired value by adjusting the Variac. Temperatures were read by means of triple-juncture copper-constantan thermocouples constructed of 40-gage wire, having an ice-and-water cold junction, and calibrated against the boiling point of nitrogen, the sublimation point of carbon dioxide, and the freezing point of water. The procedure employed during the analysis of samples in this apparatus was essentially the same as that described by Ward, except that the temperatures and pressures shown in Table II were used for the separation of the various components in a mixture rather than the values given by Ward.

In actual practice, it was found convenient to start withdrawing each component while the temperatures were lower than required for the separation, and to warm the condensers gradually to the desired temperatures while the distillation was in progress. Before the separation of any component was considered complete, however, the pressure in the condenser train was reduced to the value shown in Table II while the condensers were maintained at the proper temperature gradient.

Analyses of synthetic mixtures consisting of various amounts of ethane, propane, butanes, and isopentane in the modified Ward apparatus have demonstrated (Table III) that saturated light hydrocarbon mixtures containing no component heavier than isopentane can be analyzed with an average accuracy of about ±0.3 mole per cent or less for each component, regardless of the relative quantity of the component in the mixture.

No attempt has been made in this laboratory to separate in this apparatus either isomers or unsaturates from saturates containing the same number of carbon atoms; however, in view of the relatively small values of alpha for such separations, it is doubtful if satisfactory fractionation could be achieved. Since the butane cut is the only fraction obtainable in this apparatus in which isomers may be present, a dew-pressure apparatus has been developed for the analysis of saturated C_4 fractions from the modified Ward apparatus and is described below.

It has also been demonstrated (Table IV) that light hydrocarbon samples containing unsaturates can be separated into groups of the same number of carbon atoms in the modified Ward apparatus with about the same degree of accuracy that is realized in the analysis of saturated mixtures. Although an adequate variety of pure, unsaturated hydrocarbons was not available for synthetic blends, the effect of unsaturates upon the fractionating efficiency of the apparatus was determined by analyzing identical refinery samples containing unsaturates, one analysis being made of the straight sample and another of a hydrogenated portion of the same sample (correcting in the latter case for hydrogen).

In view of the fact that hydrocarbon samples containing unsaturates can be accurately separated into groups of the same number of carbon atoms in the modified Ward apparatus, a further investigation has been made of the possibility of determining the unsaturate content of segregated fractions from the apparatus by absorption in auxiliary equipment. Because of the small sample volumes (about 200 gaseous ml.) employed for an analysis, it was necessary to choose an apparatus for the unsaturate determinations in which gas samples of 10 ml. or less could be analyzed accurately. A Haldane-Henderson gas analysis apparatus was selected for this investigation; a 40 per cent solution of potassium hydroxide was used as the confining liquid in the buret, and one-fourth saturated bromine solution (containing 50 grams per liter of potassium bromide) was used as the absorption reagent in the pipet.

Analysis of several refinery light hydrocarbon samples containing unsaturates have indicated (Tables V and VI) that such samples can be analyzed satisfactorily by determining the unsaturate content of segregated fractions from the modified Ward apparatus by absorption in one-fourth saturated bromine water. Although no synthetic mixtures were used in this phase of the investigation, and consequently no direct proof of the accuracy of this method of analysis for unsaturates is as yet available, the fact that the total unsaturate content of each sample, as determined by hydrogenation, checked well with the total unsaturate content calculated from the bromine absorption of the segregated cuts (Table V) indicates that this method is comparable in accuracy to the hydrogenation method for determining unsatu-

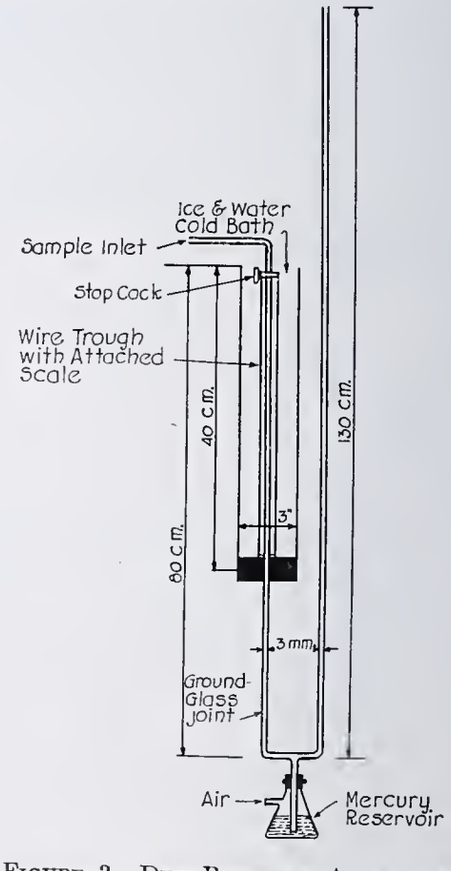

Figure 3. Dew-Pressure Apparatus

Table V. Combined Modified Ward and Haldane-Henderson Apparatus Analyses

	Run 1	Run 2
Methane	....	61.0 ± 0.2
Ethene	0.4 ± 0.2	3.5 ± 0.2
Ethane	9.4 ± 0.2	25.3 ± 0.2
Propene	21.1 ± 0.2	3.1 ± 0.2
Propane	68.3 ± 0.2	7.1 ± 0.2
Butanes	0.8 ± 0.2	
Total unsaturates:		
By hydrogenation	21.7 ± 0.2	6.7 ± 0.2
By bromination	21.7 ± 0.1	....
By bromination of segregated fractions	21.6 ± 0.1	6.6 ± 0.1

Table VI. Automatic Podbielniak and Combined Modified Ward (and Dew-Pressure or Haldane-Henderson Apparatus) Analyses

	Automatic Podbielniak Column		Modified Ward Apparatus and Auxiliary Equipment	
	Run 1	Run 2	Run 1	Run 2
Methane	60.9 ± 0.2	....	61.0 ± 0.2	....
Ethene (estimated)	3.6 ± 0.2	....	3.5 ± 0.2	....
Ethane	25.6 ± 0.2	....	25.3 ± 0.2	....
Propene	9.4 ± 0.2	....	3.1 ± 0.2	....
Propane	....	2.1 ± 0.2	7.1 ± 0.2	2.6 ± 0.2
Isobutane	0.5 ± 0.2	83.0 ± 0.2	....	83.0 ± 0.2
n-Butane	....	14.9 ± 0.2	....	14.4 ± 0.2

rates in the C_2 and C_3 fractions. The relative merits of various absorption reagents for olefin determination are discussed below.

No operating difficulties were experienced when analyzing samples in the combined modified Ward and Haldane-Henderson apparatus. Approximately 10 ml. of each fraction (except methane) segregated in the modified Ward apparatus were transferred by means of the Toepler pump to the absorption apparatus while the condensers in the modified Ward apparatus were being warmed to the temperatures required for the separation of the next fraction. Constant volume readings were usually obtained in the Haldane-Henderson sample buret after six passes through the reagent (each pass consuming approximately 20 seconds), although a minimum of ten passes was used for each analysis in order to ensure complete absorption.

Dew-Pressure Apparatus

In view of the fact that the accurate quantitative separation of isobutane from *n*-butane is a difficult and time-consuming operation and often gives undependable results when attempted in ordinary microfractionation columns, and since such a separation would probably be impracticable if attempted in a high-vacuum apparatus, an investigation has been made of methods of analysis for isobutane–*n*-butane mixtures which are based on differences in the physical properties of the gases but do not depend upon their quantitative separation. Of the two such physical methods most applicable to routine laboratory work (determination of the refractive index of the liquid mixture and determination of the dew-point pressure of the gaseous mixture), only the dew-point pressure method has been investigated in this laboratory.

Several investigators, notably Hachmuth (*2*) and Woog, Sigwalt, and Gomer (*16*), have developed apparatus for determining the dew pressures of isobutane–*n*-butane mixtures at 0° C. The dew-pressure apparatus developed during the course of this investigation (shown in Figure 3) consists simply of a Y-type manometer connected to a mercury reservoir, with half of one arm of the manometer immersed in an ice-and-water bath. The ice-and-water bath is constructed of 7.5-cm. (3-inch) glass tubing and is connected to the manometer by means of a rubber stopper. In order to read the mercury level in that portion of the manometer surrounded by the bath, a scale is attached to the back of a metal trough which is placed around the manometer arm and extends to the wall of the bath. This scale is calibrated with respect to the scale on the other arm of the manometer by raising the mercury in both arms under atmospheric pressure and taking blank readings on both scales.

The following procedure is followed during the analysis of an isobutane–*n*-butane mixture in this apparatus:

After evacuating the apparatus and connecting lines to an absolute pressure of 0.001 mm. or less and filling the bath with chipped ice and water (the temperature in the bath must be maintained at 0° C. throughout an analysis by agitating the ice-water mixture and adding ice if necessary), enough sample is admitted through the stopcock into the left arm of the manometer so that the mercury level in the sample arm of the manometer is about even with the bottom of the ice-and-water bath under atmospheric pressure. The mercury in the manometer is then raised in increments of 5 to 10 mm. by increasing the air pressure in the mercury reservoir (by means of a pressure-regulating valve), allowing sufficient time after each increase for the temperature of the sample to readjust itself to 0° C.

The dew pressure is determined by plotting the pressure on the sample in millimeters of mercury, against $\Delta V/\Delta P$, $\Delta V/\Delta P$ being the ratio of the incremental decrease in sample volume (plotted as the incremental increase in the height of mercury in the sample side of the manometer) to the incremental increase in pressure on the sample for each rise of mercury in the manometer. This ratio, which remains nearly constant as long as the sample remains in the gaseous phase, increases rapidly as the dew pressure is reached and passed. The dew pressure is taken as the point where the change in slope of the pressure *vs.* $\Delta V/\Delta P$ curve begins, and the composition of the sample is computed from the formula:

$$Y_1 = \frac{P_1}{\pi}\left(\frac{\pi - P_2}{P_1 - P_2}\right) \qquad (1)$$

where

Y_1 = mole per cent of isobutane in vapor in equilibrium with liquid at dew pressure
P_1 = vapor pressure of isobutane at 0° C. (1190 mm. of mercury)
P_2 = vapor pressure of *n*-butane at 0° C. (770 mm. of mercury)
π = total pressure on system at dew point, mm. of mercury

A somewhat simpler method of obtaining the dew pressure would be to plot the pressure, in millimeters of mercury, on the sample against the decrease in volume (plotted as the increase in the height of mercury in the sample side of the manometer) and extrapolate the lines plotted from the points obtained before and after the dew pressure is passed until they intersect. This would, however, necessitate the use of an empirical table in which the dew pressures corresponding to various percentages of isobutane (as determined by the dew-pressure analyses of synthetic mixtures) were given; such a table has not yet been developed in this laboratory.

The vapor pressure values used in Equation 1 differ somewhat from some of the values quoted by Sage and Lacey (*11*, *12*, obtained by extrapolation), Dana (*1*), and Woog, Sigwalt, and Gomer (*16*). Since these literature values differ by as much as 10 to 15 mm. for both isobutane and *n*-butane, and there seemed to be no way of selecting the correct vapor pressures from these data, the vapor pressures for the butanes were determined experimentally in this laboratory by the method described above. In view of the accurate results obtained by the dew-pressure method on synthetic mixtures when using these values, it appears that the experimentally determined vapor pressures are very nearly correct. After the dew pressure has been determined, and before the composition of the sample is calculated from Equation 1, it is necessary to correct the manometer readings at the dew pressure to standard conditions. By taking an imaginary

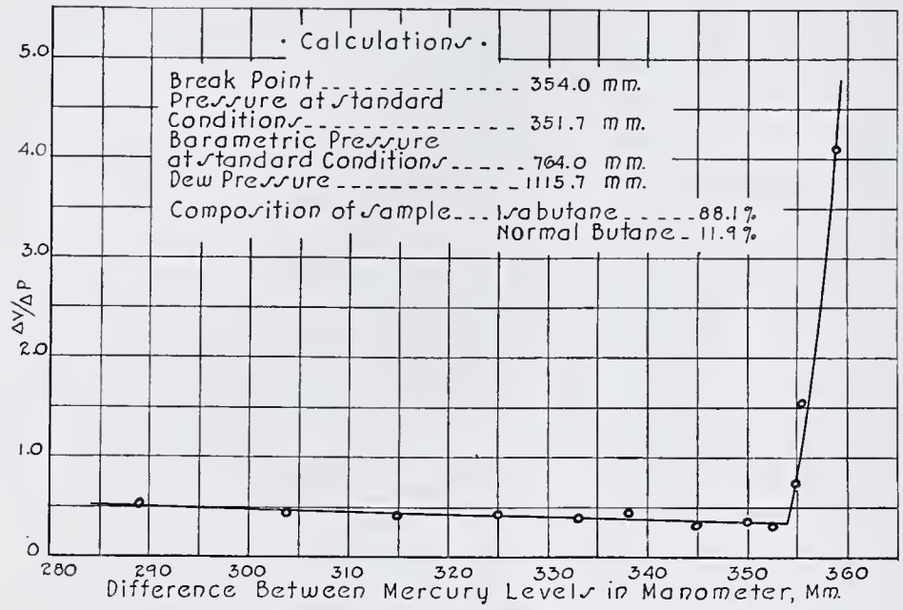

FIGURE 4. DEW-PRESSURE CURVE AND CALCULATIONS

reference plane through the bottom of the ice-and-water bath on the dew-pressure apparatus (Figure 3), the following temperature correction formula may be derived:

$$P_0 = H_R K - H_L$$

where

P_0 = corrected dew pressure, mm. of mercury
H_L = height above reference plane of mercury in left arm (sample side) of manometer at dew pressure, mm.
H_R = height above reference plane of mercury in right arm of manometer at dew pressure (equal to H_L plus difference in mercury heights in manometer at dew pressure), mm.
K = coefficient of reduction for correcting mercury heights at room temperature to 0° C.

A typical dew-pressure curve and calculations are shown in Figure 4.

Analyses of synthetic isobutane–*n*-butane mixtures by the dew-pressure method have resulted (Table VII) in an average analytical accuracy of about ±0.5 per cent (about the limit of experimental accuracy for this method of analysis). From an inspection of Equation 1, however, it is evident that the inclusion of even small amounts of higher- or lower-boiling impurities with the butanes would have an appreciable effect upon the accuracy of the analysis. In order to ascertain quantitatively the effects of small quantities of impurities upon the accuracy of the dew-pressure method of analysis, several synthetic samples containing small amounts of either propane or isopentane in addition to the butanes were analyzed by dew-pressure determinations, the composition of the samples being calculated on the assumption that no gases other than the saturated butanes were present. It was found (Table VIII) that the presence of small quantities of either gas caused errors in the analysis about three times greater than the magnitude of their concentrations. (In the case of very small concentrations of impurities, the error could not be determined accurately because the probable experimental error in blending the synthetic mixtures was about ±0.2 mole per cent for each component; however, the errors introduced by less than 0.2 per cent of an impurity are probably within the limit of experimental accuracy of the dew-pressure determinations.) It is also evident that the extent to which these impurities affect the dew-pressure determination is dependent not only upon the concentration of the impurity but also, to a lesser extent, upon the proportion of isobutane to *n*-butane in the sample.

In view of the marked effect of higher- and lower-boiling impurities upon the accuracy of the dew-pressure determination for isobutane–*n*-butane mixtures, it is evident that, in order to obtain reliable results from the analysis of hydrocarbon samples containing components other than the butanes, the butane fraction must be segregated from the other constituents of the mixture in such a way that it contains substantially no impurities. In this laboratory, no attempt has yet been made to segregate impurity-free C_4 fractions from a Podbielniak distillation; however, it is doubtful if a pure fraction could be obtained from a column distillation unless the cut were collected well after the propane-isobutane break and before the *n*-butane–isopentane break. Although, in such a procedure, the accuracy of the dew-pressure determination would still depend to a large extent upon the fractionating efficiency of the column, it is probable that a considerable saving in time and liquid nitrogen would be realized (over attempting the isobutane–*n*-butane split on the Podbielniak-type apparatus).

The feasibility of segregating saturated butane mixtures from samples containing propane and air in addition to the butanes in the modified Ward apparatus has been demonstrated in this laboratory with synthetic mixtures; analyses of such mixtures in the combined modified Ward and dew-pressure apparatus have resulted in average deviations from the true composition of about ±0.6 mole per cent or less for each component in the mixture (Table IX). Inasmuch as propane-butanes and butanes-isopentane separations are achieved in the modified Ward apparatus with about the same degree of accuracy, and small amounts of isopentane have about the same effect (although in the opposite direction) as equal amounts of propane upon the accuracy of dew-pressure determinations, it appears reasonably certain that saturated butane cuts, when properly segregated in the modified Ward apparatus, do not contain sufficient quantities of these impurities to affect materially the accuracy of the dew-pressure determination.

Although no attempt has been made to determine experimentally the effects of C_4 unsaturates upon the accuracy of dew-pressure determinations, it is obvious that appreciable quantities of these unsaturates would introduce a considerable error into the analysis. When analyzing samples containing unsaturates, therefore, it is necessary to remove the unsaturates by absorption or to saturate them by hydrogenation before analyzing them by the dew-pressure method. The practicability of analyzing hydrogenated samples in the combined modified Ward and dew-pressure apparatus has been demonstrated by numerous analyses of routine samples.

TABLE VII. DEW-PRESSURE ANALYSES OF ISOBUTANE–*n*-BUTANE MIXTURES

	Run 1	Run 2	Run 3	Run 4
	Mole per cent			
	Synthetic Mixture			
Isobutane	84.3 ± 0.2	56.3 ± 0.2	49.4 ± 0.2	19.5 ± 0.2
n-Butane	15.7 ± 0.2	43.7 ± 0.2	50.6 ± 0.2	80.5 ± 0.2
	Analysis			
Isobutane	84.4 ± 0.4	56.8 ± 0.4	49.8 ± 0.4	19.3 ± 0.4
n-Butane	15.6 ± 0.4	43.2 ± 0.4	50.2 ± 0.4	80.7 ± 0.4

Table VIII. Effect of Impurities upon Dew-Pressure Analyses

	Synthetic Mixture					Analysis				
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 1	Run 2	Run 3	Run 4	Run 5
	Mole per cent					*Mole per cent*				
Propane	.....	.....	.....	0.2 ± 0.2	0.5 ± 0.2	.....	.....	.....	.....	.....
Isobutane	49.8 ± 0.2	48.6 ± 0.2	49.6 ± 0.2	89.9 ± 0.2	81.3 ± 0.2	49.4 ± 0.4	46.5 ± 0.4	43.3 ± 0.4	90.5 ± 0.4	82.8 ± 0.4
n-Butane	49.9 ± 0.2	50.0 ± 0.2	47.7 ± 0.2	9.9 ± 0.2	18.2 ± 0.2	50.6 ± 0.4	53.5 ± 0.4	56.7 ± 0.4	9.5 ± 0.4	17.2 ± 0.4
Isopentane	0.3 ± 0.2	1.4 ± 0.2	2.7 ± 0.2	.....	.....	.....	.....	.....	.....	.....

Table IX. Combined Modified Ward and Dew-Pressure Analyses

	Synthetic Mixture			Analysis		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
	Mole per cent			*Mole per cent*		
Propane	3.9 ± 0.2	4.1 ± 0.2	17.1 ± 0.2	4.1 ± 0.2	10.0 ± 0.1	17.1 ± 0.2
Isobutane	80.3 ± 0.2	80.8 ± 0.2	59.7 ± 0.2	80.9 ± 0.4	81.1 ± 0.4	59.0 ± 0.4
n-Butane	15.8 ± 0.2	9.2 ± 0.2	23.2 ± 0.2	15.0 ± 0.4	8.9 ± 0.4	23.9 ± 0.4

Comparison of Standard Microfractionation Columns with High-Vacuum Analysis Apparatus and Auxiliary Equipment

In view of the wide differences between the types of apparatus discussed above, it might be well to compare these apparatus with respect to accuracy, speed and economy of operation, versatility, and adaptability to routine laboratory operation. Although final conclusions regarding the relative merits of the automatically controlled Podbielniak column, modified Ward apparatus, and dew-pressure apparatus cannot be made until each apparatus has been further tested with synthetic mixtures under routine laboratory operating conditions, the following comparisons, on the basis of data obtained thus far, appear to be justified.

Accuracy. When properly operated, the automatic Podbielniak Model L type column and combined modified Ward and dew-pressure apparatus are comparable in accuracy (about ±0.5 per cent or less maximum deviation from the true composition on any component through isopentane) in the analysis of saturated light hydrocarbon mixtures. Although samples containing unsaturates can be satisfactorily separated into groups of the same number of carbon atoms in either apparatus, individual olefins must be determined by analyzing the segregated fractions in auxiliary equipment. Unsaturates must be removed from C_4 fractions before satisfactory analyses for isobutane or *n*-butane can be made in either apparatus.

Operability. Consistently accurate analytical results from either apparatus are dependent to a large extent upon the proper condition and operation of the apparatus. Operating conditions for the modified Ward and dew-pressure apparatus are standardized and, after a little practice, easily followed; the establishment of optimum operating conditions in the automatic column depends to a large extent upon the skill of the operator and the mechanical condition of the equipment.

Speed and Economy of Operation. The time and liquid nitrogen required for an accurate analysis in the combined modified Ward and dew-pressure apparatus are, for nearly every type of sample, considerably less than for a comparable analysis in the automatic Podbielniak (Model L) column; this is especially true of the analysis of butane samples.

Versatility. Both the microfractionation column and the modified Ward and dew-pressure apparatus have certain limitations in the analysis of light hydrocarbon mixtures. Since, in the modified Ward apparatus, the original sample and all the individual components thereof are measured as gases, no sample containing appreciable amounts of any hydrocarbon heavier than isopentane—i. e., liquid under ordinary atmospheric conditions—can be analyzed without the use of auxiliary equipment. It would, of course, be possible to operate the apparatus in conjunction with a calibrated topping still, analyzing the overhead by fractional condensation and, if desirable, analyzing the residue by a column distillation.

On the other hand, it is possible to analyze small gaseous sample volumes (200 ml. or less) in the modified Ward and dew-pressure apparatus; samples of at least 1500 ml. (gaseous) and preferably several times larger are required for a column analysis. Small amounts of individual components, especially if they are the lightest or heaviest components of a mixture, are detected and measured much more easily in the modified Ward apparatus than in a column distillation.

In this laboratory, it has often been found convenient to operate the combined modified Ward and dew-pressure apparatus in conjunction with a microfractionation column for some types of samples.

For example, a cracked gas sample containing a small proportion of butanes-butenes is fractionated in a column; the butanes-butenes cut, along with some propane and isopentane, is segregated from the other components of the sample. This cut is then hydrogenated, the C_4 hydrocarbons are separated from the hydrogen, propane, and isopentane in the modified Ward apparatus, and the iso–*n*-butane ratio is determined in the dew-pressure apparatus. From the dew-pressure analysis, the hydrogenation of the total C_4 cut, and an analysis for isobutene (by the anhydrous hydrochloric acid method or by absorption) the amount of isobutane, isobutene, *n*-butenes, and *n*-butane in the sample may be determined with a far greater degree of accuracy than would have been possible by the column analysis alone.

Determination of Total Unsaturates in Gaseous Samples

Among the available methods for the determination of total unsaturates in gaseous samples are absorption in saturated bromine solution, absorption in solutions containing bromine in the form of a complex salt, absorption in various concentrations of sulfuric acid, and catalytic hydrogenation at room temperatures and atmospheric pressures according to the procedure developed by McMillan *et al.* (*7*).

Because bromine reacts rapidly and completely with gaseous unsaturated hydrocarbons, this reagent is often used for the determination of total unsaturates. However, it is apparent that saturated bromine solution as it is usually prepared (containing excess free bromine) attacks isobutane and, to a lesser extent, *n*-butane and therefore does not give accurate determinations of unsaturate content. It was found (Table X) that on refinery butane-butenes samples (which had been analyzed previously by hydrogenation), three passes into the reagent gave approximately correct results, whereas determinations in error by 20 per cent or more were experienced if nine or more passes were used. Reproducible results were, however, obtained by using one-fourth saturated bromine solutions (with and without excess potassium bromide, or containing mercuric nitrate) as the absorption reagent. These solutions do not appreciably attack saturated butanes, as indicated by the fact that approximately the same un-

TABLE X. COMPARATIVE ANALYSES FOR TOTAL UNSATURATE CONTENT OF PLANT SAMPLES USING BROMINE SOLUTIONS

Absorption Reagent	Number of Passes through Reagent	Total Unsaturates Found by Analysis
		Mole per cent
Saturated bromine solution	3	10.4 ± 0.1, 10.7 ± 0.1
	6	11.3 ± 0.1, 11.8 ± 0.1
	9	12.4 ± 0.1, 12.6 ± 0.1
	3	10.8 ± 0.1
	9	14.8 ± 0.1
	15	17.7 ± 0.1
One-fourth saturated bromine solution	12	34.1 ± 0.1
	15	34.5 ± 0.1
	40	34.3 ± 0.1
One-fourth saturated bromine solution containing excess KBr	12	34.1 ± 0.1
	12	34.7 ± 0.1
	12	34.8 ± 0.1
	12	34.8 ± 0.1
	16	35.4 ± 0.1
	17	35.7 ± 0.1
	22	35.7 ± 0.1
	22	35.8 ± 0.1
	40	35.2 ± 0.1

saturate content was found after twelve to forty passes through the reagent (Table X). In addition, these reagents failed to react with pure gaseous isobutane. It is believed that failure to obtain exactly reproducible results with dilute bromine solutions is attributable to the absorption of small quantities of saturated components in the liquid dibromides formed from the reaction of bromine with unsaturates.

In general, sulfuric acid solutions are not desirable reagents for the determination of total gaseous unsaturates. These reagents (especially concentrated solutions) apparently dissolve different amounts of saturated hydrocarbons, depending upon the types and concentrations of unsaturates absorbed, and either high or low analytical results are possible (Table XI). In the absence of ethene, fairly accurate results are obtainable when using 86 per cent sulfuric acid as the absorption reagent (Table XI), but the procedure is time-consuming (sixty to eighty passes through the reagent are necessary in order to obtain constant readings).

The McMillan catalytic hydrogenation method for the determination of total gaseous unsaturates was found to be both rapid and accurate in the analysis of synthetic butanes-butenes mixtures (Table XI), provided suitable correction factors were applied to compensate for the deviations of the various hydrocarbons from the perfect gas laws. Five passes over the nickel catalyst were usually sufficient to react all the unsaturates in the majority of the samples, and complete reaction was obtained in all cases after ten passes. This method possesses the disadvantage, however, that the nickel catalyst employed is easily poisoned and must be activated frequently. In order to determine the state of activation of the catalyst, frequent analyses of samples of known unsaturate content should be made.

Determination of Isobutene in Gaseous Samples

Absorption methods for the determination of isobutene in gaseous samples usually employ 60 to 70 per cent sulfuric acid, or sulfuric acid solutions, as the absorbing medium. In all such procedures, the reagent usually absorbs small quantities of *n*-butenes and butadienes in addition to isobutene, thus necessitating the employment of correction factors to compensate for errors introduced by the absorption of the *n*-olefins and diolefins. Such corrections are usually applied by passing the sample through the reagent until a constant rate of absorption per pass is obtained, and extrapolating the curve of "per cent absorbed *vs.* number of passes" back to 0 pass (Figures 5 and 6).

In this laboratory, the following reagents were investigated: 60 per cent sulfuric acid (2-minute passes), 60 per cent sulfuric acid saturated with copper sulfate (2-minute passes), and 65 per cent sulfuric acid containing 0.5 per cent by volume of *tert*-butyl alcohol (20- and 45-second passes). Analyses of synthetic mixtures by each of these procedures have shown (Table XII and Figures 5 and 6) that comparable analytical results, accurate to within a few tenths of 1 per cent, are obtainable by each of these methods, provided the procedure is carefully carried out and a sufficient number of passes are employed. Since the rate at which isobutene is absorbed by these reagents decreases as the isobutene content of the sample decreases, it is important that a sufficient number of passes be made to ensure that the absorption values used in the extrapolation do not represent the absorption of small amounts of isobutene as well as *n*-butenes; otherwise, the curve of per cent absorbed *vs.* number of passes for samples containing appreciable amounts of *n*-butenes would become difficult to interpret. For this reason, at least twenty passes should be made before extrapolating the curve obtained in this type of analysis.

TABLE XI. COMPARATIVE ANALYSES FOR TOTAL UNSATURATE CONTENT OF SYNTHETIC LIGHT HYDROCARBON MIXTURES

Analytical Method	Isobutene in Sample	2-Butene in Sample	Total Unsaturates in Sample	Unsaturates Found by Analysis
		Mole per cent		
Absorption in 94% H_2SO_4	10.4 ± 0.2	0.0 ± 0.2	10.4 ± 0.2	10.3 ± 0.1
	0.0 ± 0.2	19.4 ± 0.2	19.4 ± 0.2	21.3 ± 0.1
	0.0 ± 0.2	20.9 ± 0.2	20.9 ± 0.2	20.1 ± 0.1
	0.0 ± 0.2	21.2 ± 0.2	21.2 ± 0.2	22.4 ± 0.1
Absorption in 86.3% H_2SO_4	0.0 ± 0.2	28.0 ± 0.2	28.0 ± 0.2	28.5 ± 0.1
	0.0 ± 0.2	28.1 ± 0.2	28.1 ± 0.2	28.9 ± 0.1
McMillan catalytic hydrogenation method	0.0 ± 0.2	30.9 ± 0.2	30.9 ± 0.2	31.0 ± 0.2
	0.0 ± 0.2	40.5 ± 0.2	40.5 ± 0.2	40.2 ± 0.2
	50.1 ± 0.2	0.0 ± 0.2	50.1 ± 0.2	50.2 ± 0.2
	41.1 ± 0.2	26.7 ± 0.2	67.8 ± 0.2	67.9 ± 0.2
	0.0 ± 0.2	93.6 ± 0.2	93.6 ± 0.2	93.1 ± 0.2

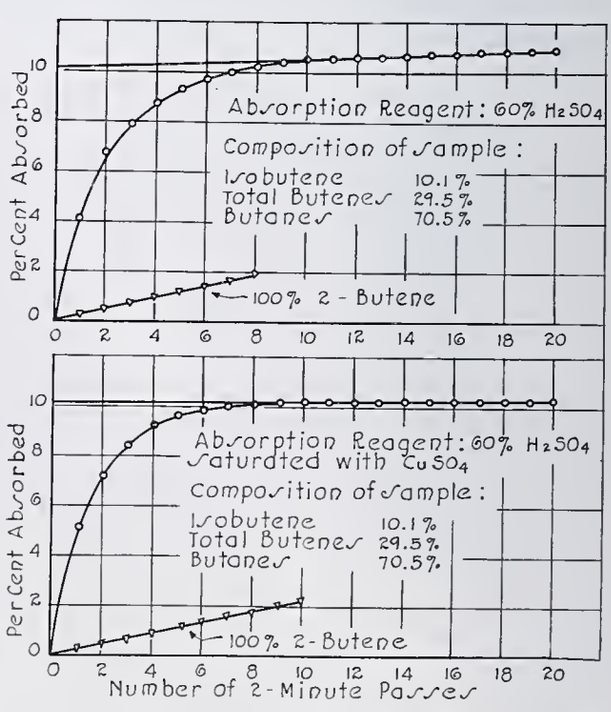

FIGURE 5. ABSORPTION OF ISOBUTENE IN 60 PER CENT SULFURIC ACID

Lower curve, acid saturated with copper sulfate

TABLE XII. COMPARATIVE ANALYSES FOR ISOBUTENE CONTENT OF SYNTHETIC LIGHT HYDROCARBON MIXTURES

Analytical Method	Composition of Synthetic Sample: Isobutene	2-Butene	Isobutene Found by Analysis
	Mole per cent		
Absorption in 60% H_2SO_4 + $CuSO_4$	10.2 ± 0.2	20.1 ± 0.2	10.4 ± 0.1
	11.6 ± 0.2	64.4 ± 0.2	11.8 ± 0.1
	19.1 ± 0.2	53.0 ± 0.2	19.2 ± 0.1
Absorption in 60% H_2SO_4 + 0.5 volume % of *tert*-butyl alcohol	6.0 ± 0.2	22.2 ± 0.2	6.1 ± 0.1
	7.3 ± 0.2	20.0 ± 0.2	7.4 ± 0.1
	7.9 ± 0.2	81.7 ± 0.2	7.8 ± 0.1
	10.1 ± 0.2	11.8 ± 0.2	10.0 ± 0.1
	21.0 ± 0.2	0.0 ± 0.2	20.9 ± 0.1
McMillan anhydrous HCl method	0.0 ± 0.2	100.0 ± 0.2	0.0 ± 0.2
	20.1 ± 0.2	79.1 ± 0.2	20.2 ± 0.2
	22.3 ± 0.2	77.7 ± 0.2	22.2 ± 0.2
	30.8 ± 0.2	62.6 ± 0.2	30.1 ± 0.2
	100.0 ± 0.2	0.0 ± 0.2	100.6 ± 0.2

Of the above reagents, the 65 per cent sulfuric acid containing 0.5 per cent *tert*-butyl alcohol was found preferable for use in routine analyses because of its greater rapidity and the smaller absorption of *n*-butenes per pass (thus permitting more accurate extrapolation).

The McMillan method (*5*) for the determination of isobutene by its reaction, in the liquid phase, with anhydrous hydrogen chloride, was found to be accurate within about ±0.2 per cent for concentrations of isobutene below about 25 mole per cent (Table XII). The reagent is, apparently, specific for isobutene, as evidenced by its failure to react with 2-butene. However, the apparatus in its original form is not well adapted to routine laboratory work because of the indefiniteness of the end point of the reaction; also, appreciable quantities of air or moisture in the sample introduce errors in the determinations. When properly operated by experienced operators, however, this method is probably superior to any of the absorption procedures now available for the determination of isobutene.

TABLE XIII. CALCULATED CHANGE IN COMPOSITION OF LIQUID SATURATED HYDROCARBON SAMPLE DURING SAMPLING FROM CONTAINER

Component	Original Sample Volume Remaining in Bomb: 100%	90%	70%	50%	30%
	Mole per cent				
Sampling under Vapor Pressure of Sample					
Ethane	10.00	9.89	9.58	9.06	...
Propane	80.00	80.08	80.30	80.65	...
Isobutane	10.00	10.03	10.12	10.29	...
Sampling by Displacement with Tap Water					
Ethane	10.00	9.99	9.98	9.94	9.89
Propane	80.00	80.01	80.02	80.05	80.09
Isobutane	10.00	10.00	10.00	10.01	10.02

Methods of Sampling and Handling Light Hydrocarbon Stocks

It has been the usual practice in many laboratories to transfer liquefied hydrocarbon samples from containers to analytical equipment by (1) withdrawing the liquid sample under its own vapor pressure, or (2) eliminating the vapor space within the bomb by water repressuring and withdrawing the liquid sample by water displacement. (It is also customary to collect liquid samples by water displacement.) Under certain conditions, described below, the employment of either of these methods may effect a change in the composition of the sample sufficient to cause measurable errors in the subsequent analysis.

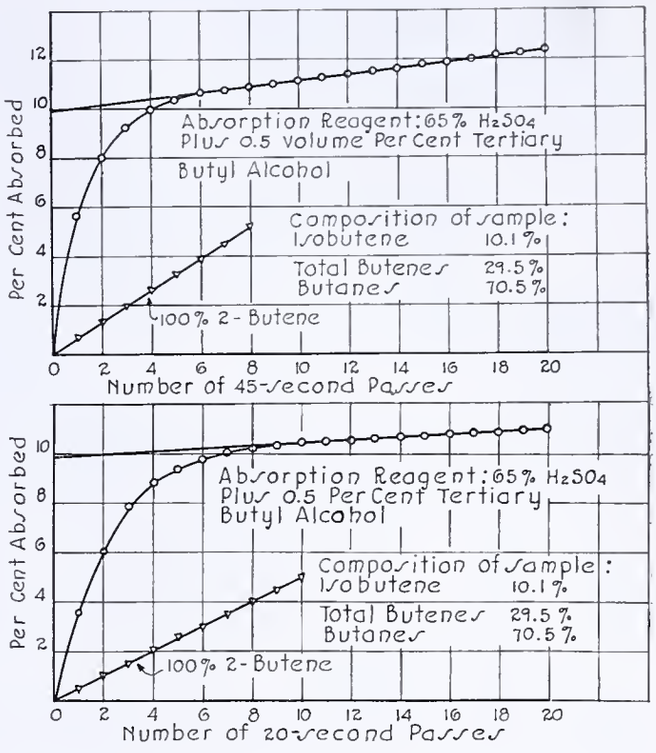
Per Cent Absorbed
Absorption Reagent: 65% H2SO4 Plus 0.5 Volume Per Cent Tertiary Butyl Alcohol
Composition of Sample: Isobutene 10.1%
Total Butenes 29.5%
Butanes 70.5%
100% 2-Butene
Number of 45-Second Passes
Absorption Reagent: 65% H2SO4 Plus 0.5 Per Cent Tertiary Butyl Alcohol
Composition of Sample: Isobutene 10.1%
Total Butenes 29.5%
Butanes 70.5%
100% 2-Butene
Number of 20-Second Passes

FIGURE 6. ABSORPTION OF ISOBUTENE IN 65 PER CENT SULFURIC ACID PLUS 0.5 VOLUME PER CENT *tert*-BUTYL ALCOHOL

TABLE XIV. CALCULATED CHANGE IN COMPOSITION OF LIQUID HYDROCARBON SAMPLE CONTAINING UNSATURATES DURING SAMPLING FROM CONTAINER

Component	Original Sample Volume Remaining in Bomb: 100%	90%	70%	50%	30%
	Mole per cent				
Sampling under Vapor Pressure of Sample					
Propene	50.00	49.99	...	49.90	49.75
Propane	50.00	50.01	...	50.10	50.25
Sampling by Displacement with Tap Water					
Propene	50.00	49.98	49.92	49.82	49.58
Propane	50.00	50.02	50.08	50.18	50.42
Sampling by Displacement with Saturated NaCl Solution					
Propene	50.00	50.00−	49.98	49.96	49.92
Propane	50.00	50.00+	50.02	50.04	50.08

When method 1 is employed, the magnitude of the error introduced depends upon several factors, including the composition of the sample, the amount of liquid withdrawn, and the size of the container. From the calculated data in Table XIII it is apparent that, when a sample consists of a mixture of hydrocarbons having widely separated boiling points, a detectable change in composition occurs when more than 10 per cent of the contents of the container is withdrawn. On the other hand (Table XIV), a negligible change in composition is experienced when more than 50 per cent of the contents is withdrawn from a bomb containing a sample whose constituents boil closely together.

When method 2 is employed, the magnitude of the error introduced depends upon the relative water solubilities of the components in the sample, the amount of water added to the bomb, and the size of the bomb. From data reported in the literature and obtained by experiment (Table XVII), it is evident that there is a wide variation in the water solubilities of the different hydrocarbons, especially between paraffins and olefins. According to calculated data based on experimentally determined solubilities (Table XIII and XIV), water repressuring causes no appreciable change in the com-

Table XV. Experimentally Determined Effect of Water Solubility on Composition of Liquid Hydrocarbon Samples Containing Unsaturates

Sample	Total Unsaturates in Original Sample	Approximate % of Bomb Filled with Water	Contact Time between Water and Sample	Total Unsaturates in Sample after Contact with Water	Reduction in Unsaturate Content
	Mole %		*Hours*	*Mole %*	*Mole %*
Refinery propane	19.1	90	24	17.8	1.3
Refinery propane	20.8	50	24	20.3	0.5
Refinery butanes	30.6	80	24	30.0	0.6
Refinery butanes	31.2	50	24	30.9	0.3

Table XVI. Experimentally Determined Solubility of Nitrogen in Tap Water and in Saturated NaCl Solution under Various Pressures

Absolute Pressure in Bomb	Bomb Temperature	Solvent	Solubility[a]	K
Atmospheres	° *C.*		*Ml./100 cc.*	
4.40	22.8	Water	7.5	0.76×10^{-4}
7.80	23.1	Water	13.6	0.78×10^{-4}
10.32	22.2	Water	18.7	0.81×10^{-4}
10.32	18.7	Saturated NaCl solution	2.15	0.09×10^{-4}

[a] Ml. of gas reduced to 0° C. and 760-mm. pressure per 100 ml. of solvent.

position of a sample consisting of saturated hydrocarbons, although the composition of samples containing unsaturates may be materially changed. Direct experiments (Table XV) on the effects of water repressuring on the composition of refinery samples containing unsaturates (made by determining, by hydrogenation, the unsaturate content of the samples before and after repressuring) have qualitatively confirmed the calculated data in Table XIV.

For many types of liquid samples, either of the above methods of transferring may cause a measurable change in the composition of the sample, especially if the sample container is small enough to necessitate the removal of a considerable percentage of its contents for analysis. Alternative methods of transferring samples are repressuring with mercury, or repressuring with a saturated solution of salt in water. Although mercury repressuring is probably the best method of transferring liquid samples contained in small bombs, no experiments have yet been made in this laboratory to determine the effect of this method upon the composition of various types of samples. Laboratory experiments have demonstrated, however (Tables XVI and XVII), that the solubility of various gases is about 80 per cent less in saturated sodium chloride solution than in water and calculations based on this fact have shown that repressuring samples with this solution results in comparatively small changes in the composition of liquid hydrocarbon samples, even when such samples contain a large proportion of unsaturates (Table XIV).

In evaluating the changes in composition of liquid hydrocarbon samples effected by the various methods of transferring such samples, an attempt has been made, whenever possible, to base all calculations upon experimental data and to check the calculated results experimentally. Calculations on the effects of water repressuring of samples were based on water-solubility values for hydrocarbons quoted in the literature and obtained by direct experiment in this laboratory.

It was assumed that Dalton's law and Henry's law (expressed in this case as $K = C/P$, where K is the solubility coefficient, C the concentration of dissolved gas in moles per 100 ml. of solvent, and P the partial pressure of the gas in atmospheres) applied to the hydrocarbon-water systems studied.

Values of K determined by experiment for the system nitrogen-water were nearly constant for pressures ranging from 4.4 to 10.32 atmospheres; values of K for various hydrocarbon-water systems were found to be slightly higher at pressures under which the hydrocarbon existed as a liquid than at atmospheric pressure (Table XVII).

The extent to which hydrocarbon-water systems followed Dalton's law was ascertained roughly by determining the water-solubility of a refinery propane-propene sample which had been analyzed in the combined modified Ward and Haldane-Henderson apparatus, and comparing the observed solubility (52.6 ml. of gas per 100 ml. of water) with the solubility calculated from the K values for the individual components in the sample (54.5 ml. of gas per 100 ml. of water).

Finally, the calculated changes in composition were checked qualitatively by observing the reduction in unsaturate content (as determined by several check hydrogenations) of several refinery samples that were repressured with various proportions of water (Table XV). It will be noted from Table XV that a detectable analytical error is introduced when 50 per cent or more of the bomb is filled with water, and that the error increases considerably as the water content of the bomb is increased.

The following procedure was employed for determining the solubilities of hydrocarbons in water and in saturated sodium chloride solution:

A 3.785-liter (1-gallon) bomb was filled with water (or solution) and approximately half the liquid was then displaced by the liquid hydrocarbon sample. The bomb, with vigorous intermittent shaking to ensure thorough contact between liquid and sample, was allowed to stand for 24 hours or more under the vapor pressure of the sample (it was found that the systems reached equilibrium within 24 hours), and then connected to an evacuated apparatus consisting of a 1-liter flask connected through a reflux condenser (through which ice water was circulated) and a calcium chloride tube to a calibrated (300-ml.) glass bulb, to which were attached a Y-type manometer and Toepler pump. A portion of the water in the bomb was then admitted to the flask and heated under partial vacuum, the evolved gases being transferred to the calibrated bulb by means of the Toepler pump and measured by noting the pressure change recorded on the manometer. Vapors were withdrawn until a constant pressure was obtained in the flask. By computing the volume of gas obtained and by weighing the flask and calcium chloride tube before and after admitting water to the system, the volume of dissolved gas per 100 ml. of water (or saturated sodium chloride solution) could be computed easily. In order to apply corrections for dissolved gases originally

Table XVII. Experimentally Determined Water Solubilities

(Liquefied hydrocarbons and mixtures of hydrocarbons under their own vapor pressure)

Sample	Absolute Pressure in Bomb	Bomb Temperature	Contact Time between Water and Hydrocarbons	Solubility[a]	K
	Atmospheres	° *C.*	*Hours*	*Ml./100 ml.*	
n-Butane	2.09	20.2	24	10.9	2.33×10^{-4}
Propane	9.43	20.9	24	27.9	1.32×10^{-4}
Refinery butane[b]	7.32	21.7	24	25.3	.....
Refinery propane[c]	13.32	22.9	24	57.1	.....
Refinery propane[c]	12.22	20.0	48	57.1	.....
Refinery propane[d]	9.16	19.8	24	52.6	.....
Isobutene	3.04	18.6	24	59.1	8.67×10^{-4}
Solubility of Isobutene in Saturated NaCl Solution					
Isobutene	2.77	17.4	72	12.1	1.95×10^{-4}

[a] Ml. of gas reduced to 0° C. and 760-mm. pressure per 100 ml. of solvent.
[b] Total unsaturates (by hydrogenation) 30.6%.
[c] Total unsaturates (by hydrogenation) 20.8%.
[d] Total unsaturates (by hydrogenation) 19.1%.

present in the tap water, blank determinations of this water were also made in the apparatus.

In computing the change in composition of various liquid light hydrocarbon samples caused by the progressive removal, under their own vapor pressure, of the samples from their containers, it was assumed that this removal consisted of a succession of small single flashes, in each of which a volume of vapor equal to the volume of liquid withdrawn was formed. By assuming Raoult's law to apply and setting up material balances between vapor and liquid after each withdrawal of sample, a series of equations was obtained which could be solved either directly or by trial and error.

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Chemical Determination of Nicotinic Acid Content of Flour and Bread

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THAT the new synthetic vitamins would ultimately be used on a major scale for the nutritional enhancement of some of our more refined foods was almost a foregone conclusion. Much emphasis has been directed toward the nutritional improvement of white flour by the restoration of the members of the vitamin B complex to the level of whole wheat. Nicotinic acid is one of the principal vitamins used in the enrichment of flour and bread, but because of limitations in the biological method for testing foods of low nicotinic acid content, no reliable data have been available up to the present time on the concentration of this vitamin in flour and related products. Previously reported chemical methods lack specificity when applied to materials of plant origin (*14*).

In the present study the Melnick-Field chemical method (*8*) for the determination of nicotinic acid has been adapted for use in analyzing flours and breads. The nicotinic acid content of a variety of such products is presented. Particular attention has been directed toward study of the reliability of the extraction procedure and of the subsequent chemical analysis, the specificity of the method for nicotinic acid in these materials, and the fate of the vitamin during the fermentation of the dough and baking of the bread.

Principle of Method

A concentrated aqueous extract is digested with hydrochloric acid to liberate any nicotinic acid from its compounds. The hydrolyzate is made alcoholic and is subjected to preferential charcoal adsorption for the removal of most of the interfering pigments without loss of nicotinic acid. The neutralized filtrate is treated with cyanogen bromide and aniline reagents, yielding a yellow color which is measured. Calculation is based on suitable correction for residual interfering color and on the increment in photometric density obtained with a known addition of nicotinic acid to an aliquot of the test solution.

Reagents

Cyanogen Bromide. Water saturated with bromine at 5° to 10° C. is just decolorized in the cold by the addition of a 10 per cent potassium cyanide solution. From 70 to 75 cc. of the potassium cyanide solution are used in the titration of 500 cc. of bromine water. This reagent when stored in the refrigerator at about 5° C. will keep almost indefinitely (more than 5 months).

Aniline Solution. Redistilled aniline is dissolved in absolute ethyl alcohol to make a 4.0 per cent solution. When stored in a brown glass bottle at room temperature, this reagent will keep for months.

Standard Nicotinic Acid Solution. This contains 100 micrograms per cc. of absolute ethyl alcohol and will keep permanently.

Buffer Solution. Composed of 1960 cc. of water, 10 cc. of phosphoric acid (85 per cent), 30 cc. of 15 per cent sodium hydroxide, and 333 cc. of absolute ethyl alcohol.

Hydrochloric Acid Solutions. Concentrated (specific gravity about 1.18), and approximately 1 *N*.

Sodium Hydroxide Solutions. Concentrated (approximately 18 *N*), and approximately 1 *N*.

Phenolphthalein Solution. 1 per cent in alcohol.

Charcoal. Darco-G-60, a vegetable charcoal (obtainable from the Darco Corp., 60 East 42nd St., New York, N. Y.).

Extraction of Nicotinic Acid

A 2-gram sample of flour or air-dried bread is weighed into a 250-cc. Erlenmeyer flask, 100 cc. of distilled water are added, and the suspension is autoclaved for 30 minutes at 7 kg. (15 pounds) pressure. The material is centrifuged and the precipitate washed twice with 50 cc. of boiling water. The pooled extract and washings may be concentrated *in vacuo* (if speed is a factor) or in an oven at 115° C. overnight to a volume of 5 cc. or less. [The distillation apparatus described by Melnick and Field (*7*) with slight modifications lends itself readily to concentration of extract. Caprylic alcohol (10 drops) is added to the pooled extracts to prevent foaming. An air-inlet tube extending to the bottom of the distillation flask controls bumping. While concentration to dryness involves no loss of nicotinic acid, subsequent

solution is facilitated by evaporating short of dryness.] Five cubic centimeters of concentrated hydrochloric acid are added and the material is substantially dissolved by immersing the flask or beaker in a boiling water bath. The solution, or suspension, is transferred to a calibrated test tube and diluted to the 15-cc. mark with water previously used to rinse the flask (or beaker). The acidity is approximately 4 *N*.

Hydrolysis, Decolorization, and Chemical Reaction

The test tube is immersed in a boiling water bath and the hydrolysis allowed to proceed for 30 to 40 minutes with occasional stirring. If foaming occurs, a few drops of caprylic alcohol may be added. The sample is cooled to room temperature and the volume restored to the original 15 cc. Ten cubic centimeters of absolute ethyl alcohol are added, and the solution is transferred to a 125-cc. Erlenmeyer flask followed by 300 mg. of charcoal. The mixture is shaken and filtered at room temperature. (Whitall Tatum filter paper, 3-inch, manufactured by the Armstrong Cork Co., Lancaster, Penna., is suitable.) A one-half aliquot of the filtrate, 12.5 cc., is pipetted into the graduated test tube, one drop of phenolphthalein is added, and the solution is neutralized in the cold to pH 7, and diluted to 15 cc. (Concentrated sodium hydroxide solution is used at first; the final pH adjustment is made with the 1 *N* reagents, using litmus paper as an outside indicator.)

For the colorimetric tests 3-cc. portions of this test solution (equivalent to one-tenth aliquots of the original sample) are used. (*a*) To the first sample 7 cc. of the alcoholic buffer solution are added. (*b*) To the second aliquot, 6 cc. of the cyanogen bromide reagent are added from a buret, followed immediately with the addition of 1 cc. of the aniline solution. The solutions are stirred after the addition of each reagent. (*c*) To the third aliquot of the test solution, 0.1 cc. of the standard nicotinic acid solution (10 micrograms) is added, followed by the reagents as in (*b*).

Table I. Nicotinic Acid Values of Aqueous Extract and Entire Test Material

Sample	Flour	Ash Content	Nicotinic Acid Values: Direct hydrolysis of sample	Nicotinic Acid Values: Hydrolysis of concentrated aqueous extract
		%	*Mg. %*	*Mg. %*
A	Whole wheat, hard, spring	1.82	9.25	6.49
B		1.84	9.60	6.20
C	Whole wheat, soft, winter	1.92	9.80	5.43
D		1.77	9.50	5.24
E	Entire wheat	0.82	4.02	2.09
F	Patent, hard (bread flour)	0.45	2.27	1.90
G		0.44	2.56	1.78
H	Patent, soft (cake flour)	0.40	2.19	1.73
I	Patent, soft (pastry flour)	0.43	2.30	1.32
J	Rye, medium	1.22	3.63	2.46
K	Rye, light	0.85	3.35	1.78
L	Rye, white	0.83	2.30	1.40
M	Clear, hard, spring	0.71	3.32	2.51
N		0.73	3.08	2.17

Readings and Calculations

The maximal yellow color, developed within 3 to 5 minutes and generally stable for at least the next 5 minutes, is read in a photoelectric colorimeter with a 420 millimicron filter. By subtracting from the photometric density of solution (*b*) that of solution (*a*) due to the color remaining after decolorization, the photometric density of the reacted nicotinic acid is obtained. This is converted into absolute units of nicotinic acid by correlating the increment in photometric density, solution (*c*) minus solution (*b*), with the amount of nicotinic acid added.

In the colorimetric measurements it is necessary to have two center settings, one for evaluating the residual color in the test solution, the other for the color developed by the chemical reaction. The photoelectric colorimeter is set to give a galvanometer reading of 100.0 (zero photometric density) with a solution containing 2 cc. of water, 1 cc. of alcohol, and 7 cc. of alcoholic buffer solution. Using the resulting center setting (No. 1, galvanometer reading with the test tube or cuvette containing the pure solution now removed), the blank solutions are read in turn to determine residual color of each of the test solutions (blank). The colorimeter is then set to give a galvanometer reading of 100.0 with a solution containing 2 cc. of water, 1 cc. of alcohol, 6 cc. of cyanogen bromide reagent, and 1 cc. of aniline solution. All the subsequent solutions containing reacted nicotinic acid are read using the new center setting (No. 2). Galvanometer readings are converted into photometric density by the formula

$$L = 2 - \log G$$

where L = photometric density and G = galvanometer reading. (Most instruments have a dual scale which allows direct reading in terms of photometric density; others are supplied with a conversion table.)

If the colorimeter cell requires more than 10 cc. (and less than 20 cc.) of solution, the alcoholic, decolorized, neutralized test solutions are diluted to double volume with a 33 per cent ethyl alcohol solution and 6-cc. aliquots are used for testing. Double quantities of reagents are also used. If in place of the test-tube type of cell, a flat cuvette is used, the center settings will be greater than 100.0 and therefore will be off the scale. In such cases the instrument is set to give a galvanometer reading of 50.0 instead of 100.0 and all readings are multiplied by 2 to give the correct value for conversion to photometric density.

Typical Calculation. Test substance = 2.000 grams of whole-wheat flour, cell = flat cuvette, and volume of solutions to be read = 20 cc.

Center setting (No. 1) = 53.5 galvanometer reading (observed) = 107.0 galvanometer reading (corrected).

Center setting (No. 2) = 57.5 galvanometer reading (observed) = 115.0 galvanometer reading (corrected).

Solution (*a*) using center setting (No. 1) = 46.5 galvanometer reading (observed) = 93.0 galvanometer reading (corrected) = 0.032 photometric density.

Solution (*b*) using center setting (No. 2) = 20.25 galvanometer reading (observed) = 40.5 galvanometer reading (corrected) = 0.392 photometric density.

Solution (*c*) using center setting (No. 2) = 9.75 galvanometer reading (observed) = 19.5 galvanometer reading (corrected) = 0.710 photometric density.

0.392 − 0.032 = 0.360 photometric density due to reacted nicotinic acid.

0.710 − 0.392 = 0.318 photometric density due to 10 micrograms of reacted nicotinic acid.

$\frac{0.360}{0.318} \times 10 = 11.3$ micrograms of nicotinic acid in test solution.

$11.31 \times 10 = 113$ micrograms in sample of test material.

$113 \times \frac{1}{2.000} = 56.5$ micrograms per gram of whole-wheat flour.

Validity of Method for Analysis of Flour and Bread

Nicotinic Acid Content. Fourteen different samples of flour were analyzed. In one series the 2-gram samples were subjected to direct acid hydrolysis according to the procedure originally reported (*8*); in the second series the samples were subjected to aqueous extraction as described above. The results are presented in Table I.

The figures obtained by direct acid hydrolysis of the test materials are from 50 to 100 per cent higher than those obtained on the aqueous extract. It was conceivable that incomplete extraction rather than increased specificity of the method for nicotinic acid was responsible for the smaller values noted in the latter series. The original procedure of direct acid treatment eliminates the possibility of low values due to faulty extraction, inasmuch as the test material is almost completely dissolved during the hydrolysis. Accordingly, both series of analyses were repeated after the addition of nicotinic acid to flours E to N, in amounts sufficient to bring the vitamin content to 5.0 milligrams per cent. In both series good recovery values were obtained, varying from 90 to 110 per cent of the added nicotinic acid.

However, a satisfactory recovery experiment does not necessarily prove that the nicotinic acid naturally present within the particles themselves has been extracted. The existence of this possibility was emphasized by experience with the chemical analysis for thiamine in foods. In order to test further the efficiency of the procedure for effecting complete extraction of nicotinic acid from within the flour granules themselves, analyses were conducted on breads baked with ordinary and nicotinic acid–enriched flours. The results are

summarized in Table II. As expected, the basal breads yielded higher values when subjected to the procedure of direct acid hydrolysis. Approximately 85 per cent of the added nicotinic acid was recovered by this method, which involves solution rather than extraction of the sample. When the tests were conducted on the aqueous extracts, smaller values were obtained, but the recovery of the added nicotinic acid was the same as that noted in the initial tests. This agreement is good evidence of the complete removal of nicotinic acid by the preliminary aqueous extraction procedure. The low recoveries of the added nicotinic acid (85 per cent) noted in testing the enriched breads are not due to errors in either method, since direct additions of nicotinic acid to air-dried bread samples are followed by complete recoveries. The smaller values obtained by the modified method appear to be due to improvement in the specificity of the procedure for nicotinic acid in plant products.

Reproducibility tests using the modified method were conducted with the fourteen flours listed in Table I and with ten ordinary and enriched breads. These have indicated that, on the average, duplicate values agree to within ±3 per cent of the mean, the maximal difference being ±7 per cent of the mean.

Table II. Completeness of Aqueous Extraction of Nicotinic Acid from Enriched Bread

Sample	Direct Hydrolysis of Sample: Found	Increment	Recovered	Hydrolysis of Concentrated Aqueous Extract: Found	Increment	Recovered[a]
	Mg./lb.	*Mg./lb.*	%	*Mg./lb.*	*Mg./lb.*	%
Basal bread A	7.9	..	..	5.7	..	..
Enriched bread[b]	16.3	8.4	83	14.5	8.8	87
Basal bread B	7.8	..	..	5.8	..	..
Enriched bread[c]	16.5	8.6	84	14.9	9.1	88

[a] Recoveries expressed as per cent of added nicotinic acid.
[b] Increment expected, 10.12 mg. of nicotinic acid per pound (assuming no loss in baking).
[c] Increment expected, 10.35 mg.

Specificity for Nicotinic Acid

This chemical reaction with cyanogen bromide and aniline is not specific for nicotinic acid alone but for the pyridine ring with the alpha position unsubstituted (*14*). The method as originally published by Melnick and Field (*8*) has received much study in the University of Wisconsin laboratory, where it gave good agreement with the black-tongue assay on dogs in the case of animal and yeast products. For this reason the simpler and less time-consuming procedure of direct acid hydrolysis is used in analyses of such products. The earlier papers (*6*, *8*) indicate what quantity of the test materials may be taken for testing and how much charcoal should be used for the decolorization. Other points are discussed, such as the stability of the reagents, the necessity for using acid in preference to alkaline hydrolysis, the validity of the method of calculation, and the necessity for carrying out the two independent blank corrections. The much higher values obtained when testing products of plant origin (*14*) can be attributed to the widespread occurrence of pyridine compounds in plant materials. The animal organism does not synthesize pyridine compounds but has a marked ability to destroy and excrete such compounds, regardless of how they are administered (*10*).

The values obtained in the present study agree very well with those found by Snell and Wright (*12*) using their microbiological assay for nicotinic acid (Table III). The fact that two totally unrelated methods, one involving a chemical reaction, the other the growth and metabolism of bacteria, yield essentially the same values, is highly presumptive that both procedures measure the same substance, and that both methods are specific for nicotinic acid in these products. More recently at the University of Wisconsin biological assay of whole-wheat flour with dogs has indicated a value of 5.4 mg. per cent (*13*), in good agreement with the values listed in Table III.

Table III. Agreement between Chemical and Microbiological Methods

Material	Nicotinic Acid Content: Chemical assay	Microbiological assay[a]
	Mg. %	*Mg. %*
Whole-wheat flour	5.2–6.5	4.5–7.0
Patent flour	1.3–1.9	1.0–2.0

[a] Method of Snell and Wright (personal communication).

Some of the published chemical methods may yield lower values than those recorded in the present paper, notwithstanding their good reproducibility and good recoveries of added nicotinic acid. However, it can be readily shown that in such cases the smaller values are not due to greater specificity for nicotinic acid but to errors inherent in the methods.

Data obtained for nicotinic acid in flour and bread by a chemical procedure which does not involve preliminary hydrolysis are unreliable. Nicotinic acid exists in nature almost entirely as free and combined nicotinamide (*4*) and in such forms reacts in variable degrees with the reagents to give colors of much smaller intensity than would be expected for an equivalent amount of free nicotinic acid (*4*, *8*). Accordingly, total nicotinic acid in enriched bread and flour cannot be evaluated under such circumstances. Only through hydrolysis to the free nicotinic acid stage can all these related compounds be converted to a common denominator and thus be capable of chemical estimation.

Improper evaluation of the blank value (residual color of the test solution) can also yield erroneous—i. e., smaller—values. The blank described by Bandier (*2*) for making this correction involves the addition of all reagents except the aromatic base to an aliquot of the test solution. However, since cyanogen bromide reacts to give the same color to an appreciable extent with nicotinic acid even in the absence of the base, false low results are obtained. It was found in tests with pure solutions containing 3 to 50 micrograms of nicotinic acid, that the yellow color developed without aniline is equivalent to 33 to 20 per cent, respectively, of that obtained when the base is included.

In other methods (*1*, *3*, *5*, *6*) the cyanogen bromide reagent is omitted but the aromatic amine included in the blank test. The base was observed to react directly with substances in the hydrolyzates to yield colors indistinguishable from that of the reacted nicotinic acid. Though this observation was confirmed, it has been shown that in the presence of cyanogen bromide these interfering side reactions do not occur (*9*). Thus, inclusion of the base in the blank test will give too high a value for the blank correction with an associated decrease in the calculated nicotinic acid content. In the earlier study (*9*) which established this point, a wide variety of test materials was investigated, but flours and breads were not included. It was of interest, therefore, to conduct comparable studies on these products, especially since the present procedure includes the use of the concentrated aqueous extract rather than the whole test material for the hydrolysis.

In Table IV representative data are presented. For convenience the procedure already reported (*8*) for making the blank correction is referred to as the "dilution blank" while the other, which includes the addition of the aromatic amine, is called the "aniline blank". (In the latter case, 6 cc. of

Table IV. Effect of Aniline in Blank in Nicotinic Acid Determination

(Indication of Specificity)

Test	Sample (2.000 Grams)	Time of Reading[a]	Photometric Density (2 − log G) Dilution blank	Aniline blank	Test with cyanogen bromide and aniline	Nicotinic Acid Using dilution blank	Nicotinic Acid Using aniline blank
						Mg. %	*Mg. %*
A	Wheat germ[b]	5 min.	0.058	0.119	0.211	6.3	3.8
		18 hours	0.051	0.248	0.105		
		48 hours	0.056	0.310	0.066		
B	Whole-wheat flour C	5 min.	0.048	0.168	0.475	5.9	4.2
		18 hours	0.051	0.426	0.168		
		48 hours	0.056	0.538	0.061		
C	Patent flour I	5 min.	0.071	0.181	0.174	1.4	0.0
		18 hours	0.071	0.420	0.100		
		48 hours	0.071	0.545	0.076		
D	Bread from ordinary patent flour	5 min.	0.046	0.155	0.194	2.1	0.6
		18 hours	0.060	0.409	0.094		
		48 hours	0.051	0.553	0.056		
						Micrograms	
E	10 micrograms of nicotinic acid	5 min.	0.000	0.000	0.323	10	10
		18 hours	0.000	0.000	0.066		
		48 hours	0.000	0.000	0.000		

[a] Reaction between nicotinic acid and reagents reaches maximum in 5 minutes.
[b] Solution from this sample was diluted 1 to 3 (1 + 2) for colorimetric tests. All readings were made on 1/10 aliquots of final test solutions.

0.03 *M* orthophosphoric acid, in addition to the aniline, were added in place of the buffer to 3 cc. of the test solution in order to simulate test conditions with respect to pH and titratable acidity.)

The data in Table IV indicate that the initial blank value (read after 5 minutes) for the solution containing aniline is in every case greater than that obtained following simple dilution to volume with the buffer. For this reason the nicotinic acid values show a marked discrepancy between the two series, depending upon which blank value is used in the calculations. In every case smaller values are obtained when the aniline blank is used.

When solutions tested for nicotinic acid are allowed to stand, there is a fading of the yellow pigment (test E, Table IV). The dilution blanks remain constant. However, the aniline blanks progressively increase in color intensity, so that at the end of 48 hours they are exceedingly large and bear no possible relationship to the solutions tested with the reagents. (Use of *p*-aminoacetophenone in an equivalent amount gave the same results.) What is especially pertinent is that in these latter tests the same solutions were used with the same amount of aniline. Apparently, the cyanogen bromide added in order to test for the nicotinic acid inhibited the aniline side reactions. Only by carrying out an independent blank determination of the residual color subsequent to decolorization, in addition to that on the reagents, can the color due to reacted nicotinic acid be evaluated.

Study of the rate of fading of the reacted nicotinic acid yields further evidence of the specificity of the present method for nicotinic acid in flour and bread. In tests with pure solutions of nicotinic acid (E, Table IV) the color produced reaches a maximum in 5 minutes, is stable for the next 5 to 10 minutes, and then slowly fades. Within 18 hours after the initiation of the reaction 80 per cent of the original color is lost and within 48 hours it completely disappears. The same rate of development and fading of color was observed in testing each of the materials described in this paper. At the end of 48 hours, the residual color in each test solution was practically the same as that for the dilution blank, indicative of complete fading of the color produced by the chemical reaction. This parallelism in the course of the reaction and in reaction velocities further supports the view that the material measured by the chemical method is actually nicotinic acid.

Fate of Nicotinic Acid during Fermentation of Dough and Baking of Bread

Using the chemical method described in this paper, analyses were conducted on aliquots of mixed bread ingredients prior to the addition of water and on the final baked loaves. Two methods of enrichment were used in these tests: the use of flour containing added nicotinic acid; and the use of ordinary flour, the extra nicotinic acid being supplied by a high-vitamin yeast. The values obtained are presented in Table V. From 83 to 99 per cent of the total nicotinic acid present in the bread ingredients was recovered on analysis of the air-dried bread samples. This small loss in nicotinic acid during the fermentation of the doughs and the baking of the breads is in keeping with the known stability of this vitamin to oxidation (*11*) and heating, even in the presence of strong acid or alkali (*10*).

Summary

The Melnick-Field method for the chemical determination of nicotinic acid has been adapted for use in testing flour and bread. The completion of the preliminary aqueous extraction, reproducibility of results, recovery of added nicotinic acid, and specificity of the method for nicotinic acid in such products have been found to be highly satisfactory.

Whole-wheat flour contains from 5.2 to 6.5 mg. of nicotinic acid per cent; patent flour from 1.3 to 1.9 mg. per cent. The more flour is refined, the lower is its nicotinic acid content. During the fermentation of dough and baking of bread only small losses of nicotinic acid occur.

Acknowledgment

The authors are indebted to Harry A. Waisman of the University of Wisconsin for suggesting the use of the aqueous extraction in analyzing plant products for nicotinic acid, and to W. A. Lohman, Jr., General Mills, Inc., New York, N. Y.,

Table V. Fate of Nicotinic Acid during Fermentation of Dough and Baking of Bread

Sample	Means of Enrichment	Nicotinic Acid Increment	Total Nicotinic Acid Values Expected	Found	Recovery
		Mg./lb. bread	*Mg./lb. bread*		*%*
A-1, white bread	None	0.0	6.1[a]	5.7	93
A-2, white bread	Nicotinic acid added to flour	10.12	16.22	14.5	89
B-1, white bread	None	0.0	6.4[a]	5.8	91
B-2, white bread	Nicotinic acid added to flour	10.35	16.75	14.9	89
C-1, white bread	None	0.0	7.0[a]	6.4	92
C-2, white bread	High-vitamin yeast	9.7	16.7	14.7	88
C-3, white bread	High-vitamin yeast	9.7	16.7	13.9	83
C-4, white bread	High-vitamin yeast	6.0	13.0	12.2	94
C-5, white bread	High-vitamin yeast	4.1	11.1	10.8	97
D-1, whole-wheat bread	None	0.0	19.7[a]	19.4	99

[a] Based on analyses of bread ingredients prior to addition of water.

and R. S. Swanson and H. S. Cullens, Thomas Baking Co., Long Island City, N. Y., for kindly supplying the flours used in this study.

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Sampling of Imported Wool for the Determination of Clean Wool Content

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RAW wool is a composite of wool fiber, grease, suint, excreta, dirt, sand, clay, vegetable matter, moisture, and occasionally paint. Its content of clean wool varies from 20 to 80 per cent, averaging around 50 per cent. In this condition it is marketed in bales or bags ranging in weight from 90 to 540 kg. (200 to 1200 pounds).

Commercially, the determination of "shrinkage", or its opposite, the percentage of clean wool content, of a lot of raw wool is based upon visual examination by specialists, an admittedly unsatisfactory procedure. When differences between the opinions of specialists exist, settlement tests are often made by scouring from 5 to 25 per cent of the bales in controversy. Sometimes one or more "representative" fleeces are drawn for test.

The accurate determination of the wool content of a lot of raw wool is of great importance to the textile industry, wool growers, the Government, and others. No completely satisfactory laboratory method has hitherto been developed, primarily because of the lack of a practical procedure for obtaining a representative sample. Several factors have contributed to this, most important being the nonuniform character of the material. The impurities are not uniformly distributed over a fleece, very wide variations from portion to portion being common. Furthermore, the percentage of "clean wool" content of the individual fleeces in a package varies more or less widely, and this is also true of the different packages in a lot. The second major difficulty is the practical one of removing portions of wool from heavy, bulky, tightly compressed bales or bags under congested conditions where the time element is of the utmost importance.

Some experimental work on the sampling of raw wool has been done by various investigators. Jones and Lush (*3*) studied the representativeness of a sample composed of 10 per cent of the fleeces, drawn at fixed numerical intervals at the time of shearing. Wilson (*7*) composited small handfuls taken from random locations in each fleece. Spencer, Hardy, and Brandon (*6*) compared the shrinkage of specific areas of fleeces with that of the entire fleeces. The Bureau of Agricultural Economics of the U. S. Department of Agriculture is conducting investigations along similar lines.

The present investigation was undertaken to determine the possibility of obtaining small samples of known and controlled levels of accuracy by the application of well-known and accepted principles of statistics.

Theoretical

RANDOM SAMPLES FROM HOMOGENEOUS LOTS. The mean of the observed values of a given quality of a number of units or small portions taken at random from a large homogeneous lot of merchandise is, in general, closer to the true average value of the quality for the entire lot than is any single observed value. The greater the number of such observations and the lesser the degree of nonuniformity among the observed values, the closer will the mean of the observations be to the true value for the lot. This

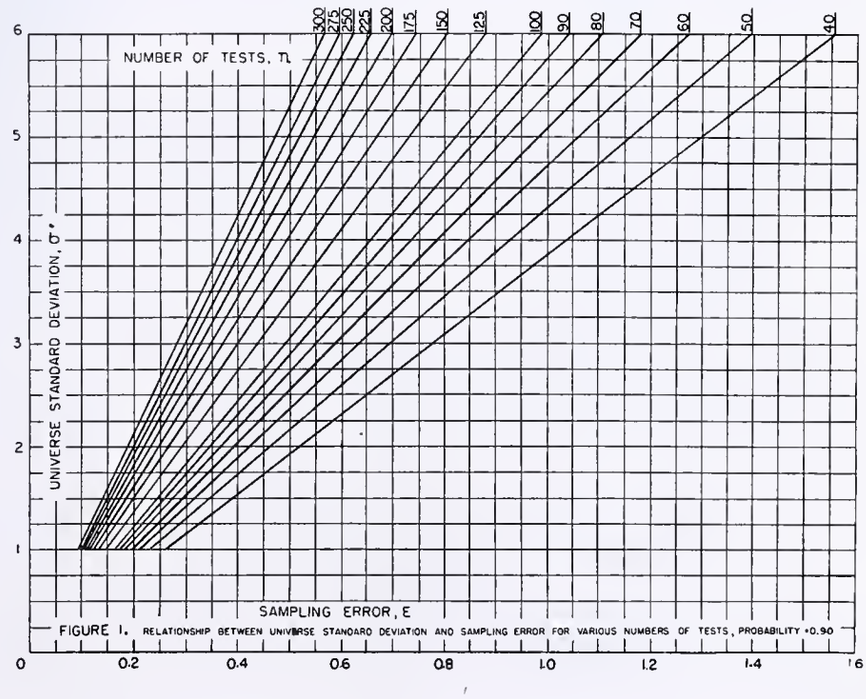

FIGURE 1. RELATIONSHIP BETWEEN UNIVERSE STANDARD DEVIATION AND SAMPLING ERROR FOR VARIOUS NUMBERS OF TESTS, PROBABILITY 0.90

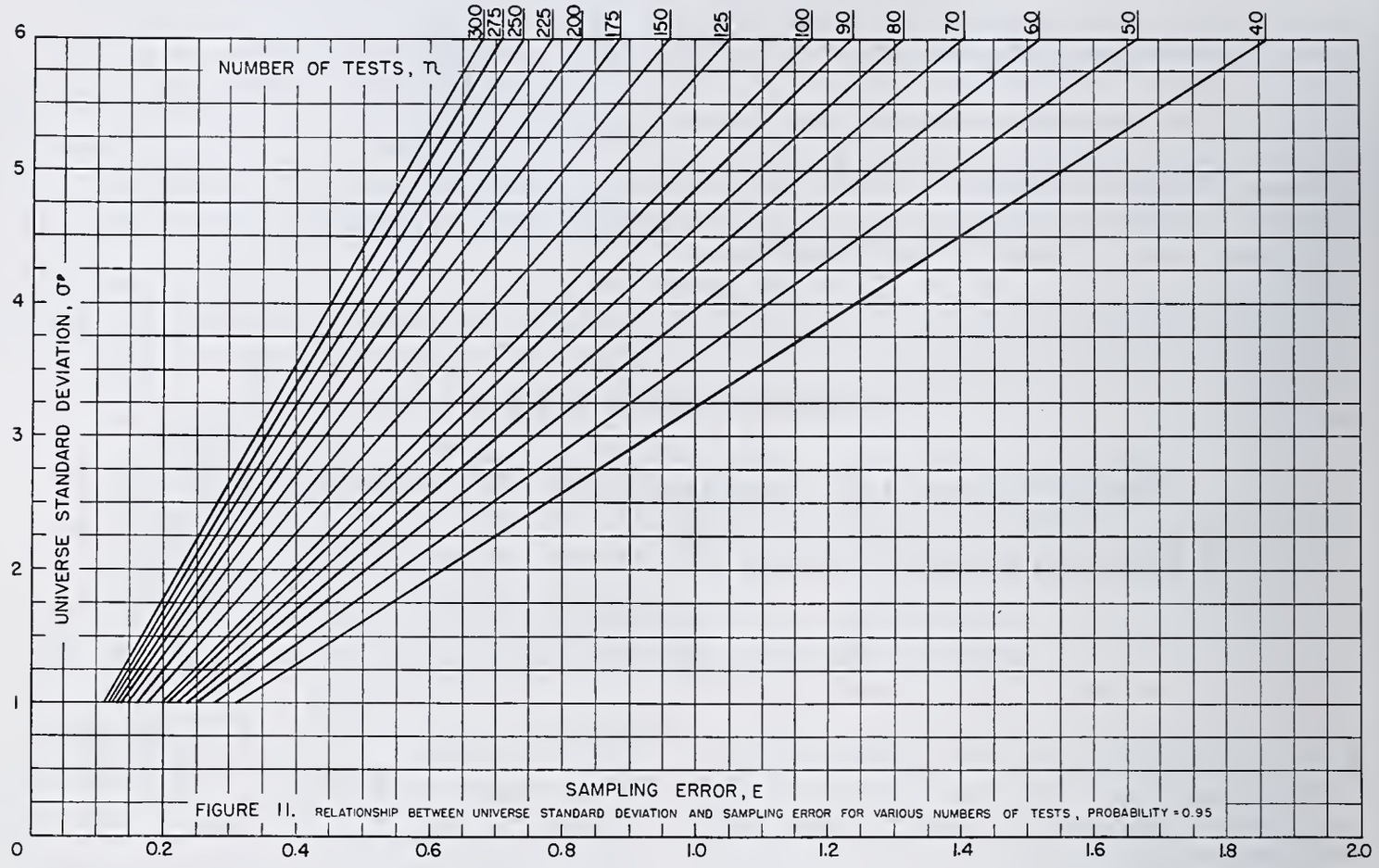

FIGURE 11. RELATIONSHIP BETWEEN UNIVERSE STANDARD DEVIATION AND SAMPLING ERROR FOR VARIOUS NUMBERS OF TESTS, PROBABILITY = 0.95

can be expressed by the following closely approximate relationship, which is based upon the theory of probability:

$$E = \frac{t\,\sigma'}{\sqrt{n}} \qquad (1)$$

where E is the sampling error, n is the number of random portions tested, and t is the probability factor. The value chosen for this factor determines the theoretical probability that the mean of the test values will not differ from the true value for the lot by more than the sampling error, E. Several such factors for various probabilities follow:

Probability	t
0.50	0.675
0.60	0.842
0.70	1.036
0.80	1.282
0.90	1.645
0.95	1.960
0.99	2.576

σ' is the universe standard deviation for all merchandise similar to the lot being sampled. The standard deviation, σ, of a set of observations is a measure of the degree of uniformity of the observations, and its value is the square root of the average of the squares of the deviations, d, of the observations from their mean, or $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$. σ' may be estimated from the average standard deviation of previous determinations or from a single sample when n is large (*1*, *2*).

Equation 1 may be used also to determine the number of test portions, n, of which a sample must consist in order that the sampling error, E, shall not exceed a specified value for a given level of probability, provided the universe standard deviation, σ', for the lot is known or can be estimated.

By the use of Figures I, II, and III, E or n may be obtained directly.

Representative Samples from Stratified Lots. A lot which is not homogeneous but is composed, instead, of a number of sublots having different mean values of the quality under consideration, or different universe standard deviations, or both, is known as a stratified lot. A "representative" sample is one drawn from a stratified lot in such a manner that each sublot is represented by a proportionate number of test portions taken at random from within the sublot.

When the universe standard deviations within the sublots are equal—i. e., $\sigma'_{w1} = \sigma'_{w2} = \ldots = \sigma'_w$—it can be shown (*4*) that for representative samples of n portions the sampling error, E, depends only upon the variability within a sublot, σ'_w, and not upon differences between the mean values of the sublots or upon the proportions of the sublots in the entire lot. Equation 1 applies, using for σ' the universe standard deviation within a sublot.

From Equation 1 it is obvious that, given the limit of permissible sampling error, E, for a specified probability, the number of random test portions, n, to be taken from a lot of material to constitute a sample thereof can be calculated, provided the universe standard deviation, σ', is known. This is practically never the case in actual practice, but a reasonably close value can be selected when sufficient previous determinations of standard deviation have been made on random samples from similar material. Any serious change of practice on the part of the producer, or variation in the nature of the material, or difference in the sampling procedure, is reflected in the changed level of the standard deviations of subsequent samples, so that necessary revision of the selected value of σ' can be made. Whatever the selected value may be, statistical procedure provides a method whereby the sampling error at any level of probability may be reliably estimated from the actual data obtained by testing the samples.

The choice of particular values for the probability and the allowable sampling error is a matter for judgment and depends upon many factors, such as the use to be made of the test results, the cost of sampling and testing, etc. A probability of 95 per cent, or chances of 95 in 100, is often used. For the sampling of lots of imported greasy wool for the determination of wool content a maximum sampling error of 0.5 per cent of wool content, with a probability of 95 in 100, is satisfactory for most purposes.

Experimental

Nature of Wool Investigated. The initial phase of this investigation was limited to Australian and South Ameri-

can wools, each of which represents a major type of imported raw apparel wool, in so far as methods of preparation and packaging are concerned. The former type includes wool not only from Australia, but also from New Zealand and South Africa; the latter type includes wool from both Argentina and Uruguay. Bales of Australian type wool contain about forty graded fleeces from which the inferior portions and the grosser impurities have been removed. The average weight is about 135 kg. (300 pounds), and average dimensions about 90 × 75 × 75 cm. (36 × 30 × 30 inches). South American bales are larger; they weigh about 450 kg. (1000 pounds) and measure approximately 150 × 90 × 75 cm. (60 × 36 × 30 inches). They are more tightly compressed than are bales of Australian wool and the fleeces are not, as a rule, so carefully prepared and graded. The outstanding characteristic of both these types of imported wool is the relative uniformity, as compared with domestic wool, of the contents of each package, reflective of the greater care with which the foreign fleeces are graded and prepared for packing. This higher degree of uniformity aids greatly the development of a practicable method for sampling imported wool.

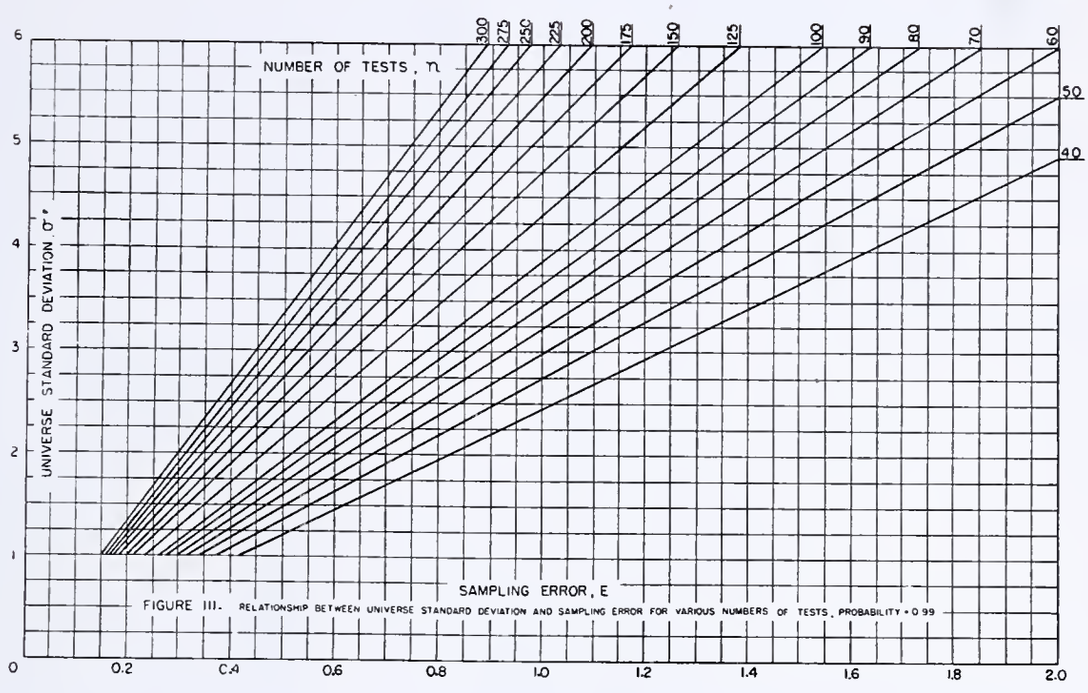

FIGURE III. RELATIONSHIP BETWEEN UNIVERSE STANDARD DEVIATION AND SAMPLING ERROR FOR VARIOUS NUMBERS OF TESTS, PROBABILITY = 0.99

Lots of Australian and South American greasy apparel wool, as imported, are usually composed of a number of sublots of one or more bales. It is generally accepted that all the bales in a sublot contain wool similar in character, quality, and grade, have the same level of percentage clean content, and have been produced, treated, and packed in substantially the same manner. Such sublots, therefore, are the "universes" to which the universe standard deviation, σ', of Equation 1 applies.

Method of Sampling Used. The experimental work was directed toward the determination of the possibilities of representative sampling of imported greasy apparel wool.

An equal number of cores (one or more) were taken from each bale by means of specially designed equipment, consisting essentially of a hand-operated, motor-driven, revolving tube provided with easily replaceable cutting edges. The locations of the borings in any bale were chosen at random, limited by the presence of baling bands or metal closure devices. Practical considerations led to the adoption of 5 cm. (2 inches) as the most suitable diameter for the tube. The maximum feasible depth of penetration was 40 cm. (16 inches); usually, however, a depth of 20 to 25 cm. (8 to 10 inches), yielding a core weighing 100 to 150 grams, was found satisfactory. Contamination of the sample with burlap was avoided by cutting a V-shaped slit in the bale covering and raising the flap before boring. Each core was immediately put into a labeled waxed paper bag which was kept in a moisture-proof drum container until weighed.

Laboratory Procedure in Determination of Clean Content. The weighed cores, in labeled cotton net bags of fine mesh, were degreased with a volatile solvent, then fluffed by hand. In this condition the wool, still in the cotton bags, was horoughly washed by suspension in warm dilute soap solutions and passage between the rolls of a wringer. During this process the bulk of the vegetable impurities was carefully removed by hand. The wool was then rinsed, hydroextracted, and dried to constant weight at 105–110° C. in electric conditioning ovens. Total extractives and ash in the dried wool were determined on test portions. Remaining vegetable matter was determined by picking it out by hand or by comparing with prepared standards. All percentages of clean wool content were computed to the same content of moisture, extractives, and ash in the final product.

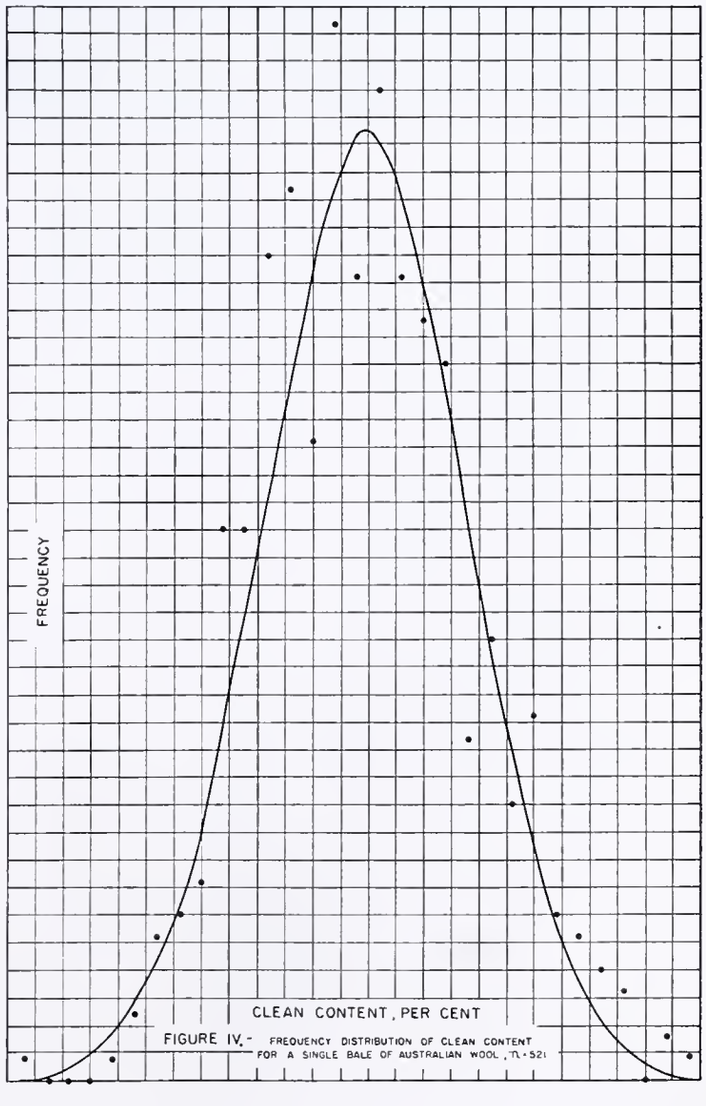

FIGURE IV. FREQUENCY DISTRIBUTION OF CLEAN CONTENT FOR A SINGLE BALE OF AUSTRALIAN WOOL, n = 521

Table I. Representative Values

Origin	No. of Bales	Clean Content	Average Weight per Core	No. of Test Portions, n	Standard Deviation, σ
		%	*Grams*		
Australia	4	48.6	96	40	2.1
	3	56.0	112	54	1.9
	1	60.2	124	18	1.6
	1	61.0	130	16	1.1
	1	62.4	110	16	1.9
	1	67.0	93	18	1.9
	1	68.6	128	18	1.2
	1	71.3	117	18	1.8
South Africa	5	54.2	145	30	2.2
Argentina	1	48.1	211	20	2.3
	1	50.4	200	20	2.8
	1	46.8	210	20	2.4
	1	48.5	218	20	1.8
Uruguay	1	57.4	162	15	1.7

Statistical Characteristics of Imported Wool. *Form of Distribution.* The probabilities associated with Equation 1 are based upon the so-called normal distribution of the means of samples about the true average. The distribution of means of samples is approximately normal no matter what the original distribution of the individual observations (*5*); however, the more nearly "normally" these observations are distributed, the more exact is the relationship.

Figure IV is typical of the distribution of the observations of percentage clean content in individual bales of Australian wool. Figure V shows the distribution for a large lot of such wool. The theoretical "normal" curves corresponding to the experimental data are shown for comparison.

Standard Deviation. Table I shows some representative values of standard deviation found for samples from single bales and from sublots of Australian and South American types of greasy apparel wool drawn with the equipment and in the manner described above. The average value for the standard deviation of the Australian wool samples was about 1.7, while that for the Argentine samples was about 2.3. For both types of wool the individual standard deviations observed for the samples (a majority comprising 15 to 20 test portions) covered a range of 1.0.

Among the factors that contribute to the variations in σ for samples of each type of wool is nonuniformity in the weight of the individual cores. The standard deviation may be expected to decrease if the weight of the test portions is increased, because the observed value of the percentage clean content of each core is in itself of the nature of an average. From the data available it appears that variations in the individual core weights not exceeding 25 per cent of the average have relatively little effect upon the value of σ. However, since it is probable that greater variations may produce significant effects, it would be necessary to specify the weight of cores and the limits of permissible variation in any standard wool sampling method based upon the statistical approach.

Experimental Verification. The applicability of representative sampling to imported wool by the procedure outlined above was studied experimentally by sampling and testing a number of lots of such wool. In these tests information was sought on reproducibility and accuracy. The distinction between precision and accuracy is well known. The former is a measure of the reproducibility of the results; it may or may not be reflective of the accuracy, depending upon the adequacy of the test methods and the homogeneity of the material with respect to the particular method of sampling used. The ultimate proof of the accuracy of a sample is a comparison of the results found by testing the sample and then by testing the entire quantity of material.

Table II shows the results of tests of triplicate representative samples from each of three lots of imported greasy wool. Table III compares the percentage clean content of representative samples from each of ten lots of wool with the actual clean content found by carefully controlled scouring and carbonizing tests in a commercial scouring plant. Strict control of the commercial tests was essential if the comparison was to be significant, since it was found that precisions of the order desired were not normally obtained in ordinary practice. As a result of the special precautions taken, the commercially determined percentage clean contents have a precision of the order of ± 0.5 per cent of clean content.

For a probability of 95 per cent, assuming a universe standard deviation, σ', of 2.0 the anticipated maximum sampling errors, E, for samples of size n = 103, 89, and 129 are ± 0.39, ± 0.42, and ± 0.35 per cent, respectively, or ranges of 0.8, 0.8, and 0.7 per cent. Reference to Table II shows that the test results are well within the computed ranges.

The agreement found between the percentage clean contents of the samples and the entire lots (Table III) is considered very good, particularly in view of the fact that the commercially determined values are not absolute, their precision

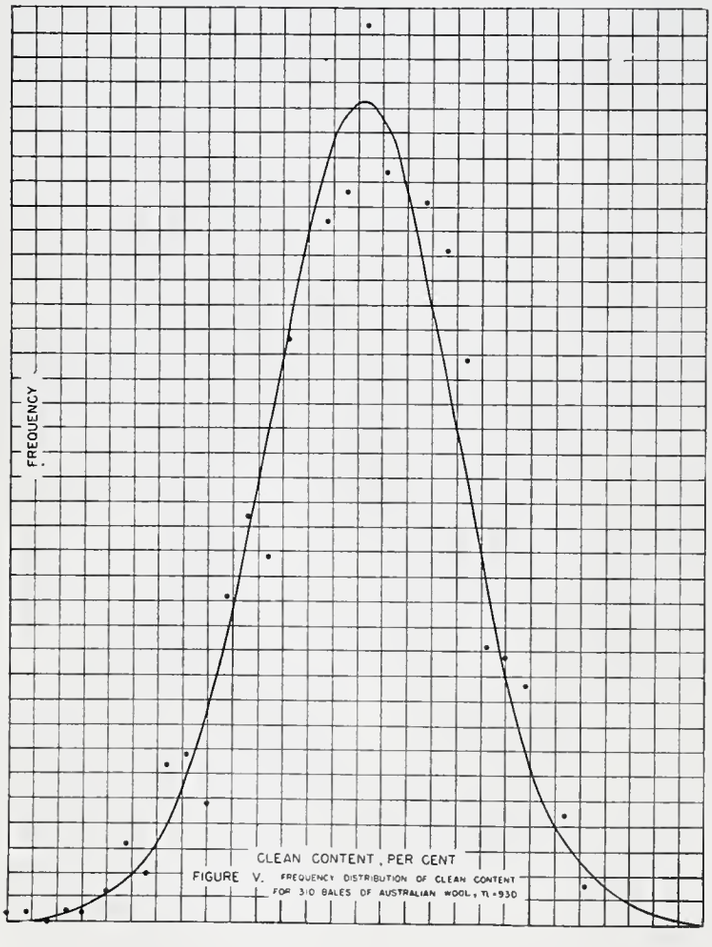

FIGURE V. FREQUENCY DISTRIBUTION OF CLEAN CONTENT FOR 310 BALES OF AUSTRALIAN WOOL, 71-930

Table II. Tests on Triplicate Samples of Imported Wool

Sample	Average Weight per Core	No. of Cores	Clean Content
	Grams		%
	From 103-Bale Lot of Australian Wool		
1	99	103	55.4
2	103	103	55.2
3	104	103	55.6
	From 89-Bale Lot of Australian Wool		
1	75	89	56.4
2	74	89	55.9
3	76	89	56.4
	From 129-Bale Lot of Cape Merino Wool		
1	105	129	50.9
2	106	129	50.7
3	101	129	50.9

Table III. Comparison of Percentage Clean Content of Samples with That of Entire Lots

Lot No.	Origin and Type	No. of Bales	Weight of Lot	No. of Cores in Sample	Average Weight per Core	Clean Content		
						Sample	Entire lot	Difference
			Pounds		*Grams*	%	%	%
1	B. A. 5's	5	4789	50	244	67.1	67.6	−0.5
2	B. A. Cotts	5	4824	75	194	71.3	71.7	−0.4
3	Aus. merino	17	4917	102	97	54.7	55.5	−0.8
4	Cape, pulled	16	4866	111	102	61.0	60.0	1.0
5	B. A. 58–60's	5	4856	50	215	51.6	51.5	0.1
6	Greasy Chubut	7	5569	70	217	41.5	41.1	0.4
7	B. A. lambs	5	4769	100	237	69.7	70.5	−0.8
8	B. A. 2nd clip	5	4805	50	234	63.1	64.3	−1.2
9	M. V. 58–60's	5	4752	50	196	57.9	57.7	0.2
10	M. V. 60–64's	5	4980	50	202	58.1	58.0	0.1
					Mean	59.60	59.79	0.55

Table IV. Uniformity of Wool in Bale

	Weight	No. of Fleeces	Average Clean Content	Standard Deviation
	Lbs.		%	%
Middle half	117	15	60.1	3.5
Outer half	114	15	59.7	4.1

being no better than ±0.5 per cent of clean content. Particularly significant is the fact that the means of the sample results and of the mill results are in practical agreement (difference less than 0.2 per cent). It indicates that in these tests the sampling method was free of significant constant errors and therefore suitable for obtaining representative samples for the determination of the clean content of the lots of wool.

Discussion

The success of the sampling method outlined is dependent upon truly random sampling within the bale. By taking cores, complete randomness is somewhat limited, because the boring always takes place from the surface of the bale to the interior, generally about one quarter of the depth. Obviously, if we define the exterior of the bale as that depth to which the tube penetrates, then if the interior of the bale were to have a constant different level of clean content than the exterior, the accuracy of the result would be affected. Such a condition did not exist in any of the bales tested in the course of this investigation. The results of the comparative tests on the ten lots of wool (Table III) give strong evidence that no significant constant difference existed between the exterior and interior of those bales.

Additional evidence of the comparative uniformity of the wool throughout a bale is afforded by the data obtained in another line of investigation of sampling methods. All the fleeces in a bale of Australian wool were removed individually and their respective clean contents determined. Those in the middle half of the bale were compared with the remainder. Table IV summarizes the results.

While uniformity in the statistical sense has been found to obtain in the past within bales of Australian and South American apparel wools, there is no assurance that temporary local conditions affecting this uniformity may not occur, or that commercial practice may not change in the future. Adequate precautions must be taken to ascertain any changes in the statistical character of the material and, if present, suitable modifications in sampling procedure must be introduced, as indicated in the suggested practice discussed below.

When the universe standard deviation, σ', for any type of wool has been established after sufficiently broad experience, and when statistical uniformity is known or judged to obtain, the individual cores need not be tested separately. Under such conditions all the cores may be composited into a single sample for the determination of the average percentage clean content. The work of laboratory testing may thus be greatly facilitated and reduced.

Suggested Practice for Sampling Imported Greasy Apparel Wool. 1. Establish the universe standard deviation, σ', for single bales, or for sublots of similar bales having the same average clean content, for each type of wool, by determining the standard deviations of a sufficient number of samples of 25 or more cores under the conditions of sampling that obtain.

2. From these data select representative values of σ' for each type of wool. Conservative judgment makes it advisable to select approximately the highest values found.

3. Decide upon the limit of permissible sampling error, E, and the probability factor, t, which meet the requirements of the use to which the test results are to be put. It should be borne in mind that sample size and, hence, sampling costs, rise rapidly with lower limits of permissible sampling error.

4. From Equation 1 or Figure I, II, or III, ascertain the number of cores, n, to be taken for a sample. Consider this number as a minimum.

5. If the lot does not consist of bales of wool approximately equal in weight, containing the same kind of wool or hair (sheep, goat, camel, alpaca, etc.) from the same country of origin, having the same general character (in fleeces, matchings, etc.) and in the same general condition (greasy, scoured, skirted, pulled, etc.), subdivide the shipment into "sampling units" in each of which these conditions are met. Consider each such unit as a separate lot for sampling and testing purposes.

6. From random locations in each bale in a sampling unit take an equal number of cores 5 cm. (2 inches) in diameter and 22.5 cm. (9 inches) long; the total number of cores should not be less than the calculated number, n. For routine tests the cores may be composited and an average percentage of clean content determined thereon.

7. Periodically redetermine the universe standard deviation, σ', to establish its constancy and continued applicability. At these times the relative uniformity of the wool within the bales should be verified. This may best be accomplished by taking a second core, by means of a longer sampling tube, from the same borings made in sampling for verification of σ'. The mean percentages of clean content and the standard deviations of such duplicate samples may then be compared for evidence of "control" (*1*, *5*)—that is, agreement within the limits of normal variation.

The procedure outlined above should be followed if many routine sampling tests are to be made. As a guide for the occasional sampling of shipments of Australian or South American apparel greasy wool the data obtained in the experimental work described in this paper may be used, provided the sampling tool and procedure described above are employed. A value of 2.5 for the universe standard deviation, σ', which is about the highest value observed in these tests, is suggested. Under these conditions the sampling error of the average of 100 cores should not exceed 0.5 per cent of clean content, approximately 95 times in 100.

Acknowledgment

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Spectrographic Studies of Coprecipitation

Fourth-Period Elements with Barium Sulfate, and Copper and Zinc with Lead Sulfate

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The coprecipitation of iron, cobalt, nickel, chromium, and manganese with barium sulfate and copper and zinc with lead sulfate was studied spectrographically. All precipitates of barium sulfate were prepared by the method of Popoff and Neuman, which consists of addition of the sulfate ions to the solution of the barium ions. It was found that cobalt and nickel do not coprecipitate with barium sulfate, whereas iron, manganese, and chromium do. Copper and zinc coprecipitate with lead sulfate.

IN QUANTITATIVE analysis it is of extreme importance that the precipitate used in the determination of any element or group be as pure as possible. In practically all cases, other ions are present in addition to the one being determined. It is, therefore, of prime importance to know whether the elements or groups present will coprecipitate with the substance being precipitated and to what extent the weight of the resulting precipitate will be affected.

Since the elements in the fourth period are frequently encountered in the determination of sulfate as barium sulfate and since lead is commonly determined as the sulfate in brasses with both copper and zinc in solution, the problem deserves careful study.

Spectrographic methods as carried out by Popoff, Waldbauer, and McCann (*8*) and Waldbauer and Gantz (*11*) were used in this study.

Materials Used

Water, sulfuric acid, and nitric acid were purified by distillation. All water used in making solutions and in recrystallizations was redistilled from alkaline permanganate solution in an all-Pyrex still having a block tin condenser. Nitric acid and sulfuric acid were distilled in a Pyrex still. Upon examination in the spectrograph, the water and acids showed no trace of the elements to be studied.

All salts were recrystallized from redistilled water to remove any impurities that were initially present.

Fifty per cent alcohol was prepared from commercial 95 per cent ethyl alcohol decanted from silver nitrate, and distilled from calcium hydroxide.

A sulfuric acid solution, made up from the distilled acid so that 40 grams of solution were equivalent to approximately 0.8 gram of barium sulfate, was used in all barium sulfate precipitations. This solution was standardized against Bureau of Standards potassium acid phthalate (*7*). The barium chloride solution was made to contain 21 grams per liter.

When the presence of any of the cations was desired as an impurity in the solution of barium chloride, solid chlorides of the metals were added instead of stock solutions, to eliminate any change, such as hydrolysis, which would make it difficult to determine exactly the quantity of metal being added. In the case of iron, ferric ammonium chloride was used because it is a crystalline substance of definite composition, whereas ferric chloride tends to hydrolyze.

The lead, copper, and zinc standard solutions were prepared by dissolving spectrographically pure metals in distilled nitric acid. Ten milliliters of each solution contained a weight of other metal equivalent to 0.5 gram of lead sulfate.

All barium sulfate precipitates for spectrographic analysis were obtained by the method of Waldbauer and Gantz (*11*). Purified salts were used in the preparation of spectrographic standards, the range of which was 0.01 to 1 per cent of each cation. The spectrographic standards had the same barium sulfate and lead sulfate content as the precipitate under examination.

Apparatus

The apparatus for the preparation and study of precipitates is essentially that used by Popoff and Neuman (*7*) and Popoff, Waldbauer, and McCann (*8*). Platinum Gooch crucibles were used in the barium sulfate precipitations, while Jena glass crucibles were used in the study of lead sulfate precipitations in order that the precipitates studied gravimetrically could be used in the spectrographic study without the danger of contamination with asbestos. It was necessary to use asbestos in the case of barium sulfate, since no Jena crucibles suitable for use with barium sulfate were available.

The graphite electrodes were found to show a slight lead contamination. Line 2614.2 Å. appeared when a photograph of the spectrum, emitted by the pure electrodes alone, was taken. This is one of de Gramont's "*raies ultimes*" for lead (*1*). No traces of the other elements involved were found.

General Methods of Analysis

BARIUM SULFATE PRECIPITATIONS. Two hundred fifty milliliters of water were added to an acidified (3 to 4 ml. of 6 *N* hydrochloric acid) solution containing a 10-ml. excess of barium chloride and that amount of cation impurity equivalent to all the barium to be precipitated as the sulfate. The barium chloride solution was kept just below the boiling point by means of a hot plate while 40 grams of the standard sulfuric acid were added dropwise with constant stirring. The precipitates were digested for one hour at 65° C. on a constant-temperature air bath. The mother liquor was decanted, and the precipitate was washed with four 15-ml. portions of water, and then washed into the crucible. The precipitates were heated for one hour at 800° C.

For spectrographic determination, the electrodes and solutions were prepared as previously described (*8*). An equal number of drops of standard, or of solution to be analyzed, were placed in each electrode, and the electrodes dried in an oven for 8 hours at a temperature of 250° to 300° C. The high temperature is necessary to dry the electrodes completely, for they do not burn well when there is even a small quantity of sulfuric acid in the graphite.

LEAD SULFATE PRECIPITATIONS. Stock solutions of the nitrates of lead, copper, and zinc were prepared. Equivalent portions of lead solution were then placed in each of four beakers. The first and third portions were analyzed for lead sulfate content merely with the addition of 10 ml. of nitric acid followed by 5 ml. of concentrated sulfuric acid. The second and fourth portions contained 10 ml. of the foreign metallic solution (either copper or zinc) added previous to the addition of the sulfuric acid.

The method of precipitation of lead sulfate was essentially the same as described for barium sulfate. The solutions containing the precipitates were evaporated on hot plates until all the nitric acid was removed. The beakers and contents were then cooled to room temperature, each watch glass was rinsed off into its respective beaker with 50 ml. of redistilled water, and the solution and precipitate were warmed gently for 5 minutes and then cooled for one hour in an ice bath. The precipitates were then filtered through the Jena crucibles, and washed with 100 ml. of 2 per cent sulfuric acid solution and then with 30 ml. of the 50 per cent alcohol solution. Finally they were dried in the oven between 130° and 140° C. for 4 hours, weighed, replaced in the oven for one hour, and reweighed. In no analysis was it necessary to dry the precipitates more than three times for them to reach constant weight.

The procedure for spectrographic determination was essentially that of Nitchie (*5*) and Popoff, Waldbauer, and McCann (*8*). The visual analysis of the plates was made with a bifocal microscope (magnifying power of ×7) using transmitted light reflected from a sheet of white paper.

Lines Used	
Copper	2961.18 and 2961.89 Å.
Zinc	3282.3, 3302.6, and 3345 Å.
Cobalt	2694 Å.
Nickel	2416 Å.
Iron	2598 Å.
Manganese	2576, 2593.7, and 2605.7 Å.
Chromium	2843.25, 2835.64, and 2830.47 Å.

Experimental Results

Of the elements in the fourth period, titanium and vanadium were not studied because of the inherent difficulties in their use, no scandium salts were available, and potassium (*7*) and calcium (*11*) had previously been studied.

The first procedure was to determine qualitatively whether or not the element in question did coprecipitate. The precipitates of barium sulfate were prepared from barium chloride solutions containing amounts of barium chloride and impurity, each equivalent to 0.8 gram of barium sulfate. Lead sulfate precipitates were prepared from solutions containing lead nitrate and impurity, each equivalent to 1.0 gram of lead sulfate. Spectrograms were taken, and the presence of elements being studied was determined by the presence or absence of the most persistent lines. The absence of the lines of the element in question was considered as conclusive evidence that the element was not present only after careful check by recognized analytical procedures.

Cobalt and nickel were the first elements studied. Mellor (*4*) states that cobalt coprecipitates with barium sulfate, and Johnston and Adams (*2*) and Lange and Berger (*3*) report that nickel coprecipitates with barium sulfate. However, neither cobalt nor nickel could be found in the precipitates by spectrographic methods. When regular qualitative methods using organic reagents were used, no trace of either element could be found. The organic reagents used were: for cobalt, nitroso-beta-naphthol and for nickel, dimethylglyoxime (*6*).

Table I. Contamination of Iron, Manganese, and Chromium in Barium Sulfate

Element	Barium Sulfate Calculated	Barium Sulfate Found	Difference from Theoretical	Element in $BaSO_4$ by Spectrograph
	Gram	*Gram*	*Mg.*	%
None	0.8236	0.8234	− 0.2	..
	0.8315	0.8317	+ 0.2	..
	0.8015	0.8015	0.0	..
	0.7860	0.7861	+ 0.1	..
			Av. + 0.025	
Iron	0.8604	0.8512	− 9.2	0.75
	0.8195	0.8096	− 9.9	0.75
	0.8624	0.8521	−10.3	0.75
	0.8128	0.8024	−10.4	0.75
			Av. −10.0	
Manganese	0.8115	0.8099	− 1.6	0.01
	0.8165	0.8146	− 1.9	0.01
	0.8244	0.8220	− 2.4	0.01
	0.8567	0.8542	− 2.5	0.01
			Av. − 2.1	
Chromium	0.8859	0.8795	− 6.4	..
	0.8075	0.8008	− 6.7	..
	0.8050	0.7975	− 7.5	..
	0.7957	0.7868	− 8.9	..
			Av. − 7.4	

In like manner iron, manganese, and chromium with barium sulfate and copper and zinc with lead sulfate were studied. Iron and manganese were found to be coprecipitated with barium sulfate. Because of the juxtaposition of other lines with the "*raies ultimes*" of chromium, the spectrographic study of the coprecipitation of this element was considered of little value. Indications, however, pointed strongly toward coprecipitation of chromium.

It was also found that copper and zinc were coprecipitated with lead sulfate. The gravimetric study, therefore, was devoted to iron, manganese, chromium, copper, and zinc; the quantitative spectrographic study was limited to iron, manganese, copper, and zinc for the reasons mentioned above.

Standards were prepared for the elements in question, and by comparison with these standards the approximate per cent of the element coprecipitated was determined. The results are recorded in Tables I and II.

Discussion

In the cases of iron, manganese, and chromium the weights of precipitates obtained were less than the theoretical, indicating that some phenomenon other than adsorption took place. This phenomenon may possibly be due to the formation of complex ions containing iron, manganese, and chromium similar to those discussed by Recoura (*9*, *10*) in which iron and chromium are in the form of "ferrisulfuric acid" and "chromosulfuric acid".

Table II. Contamination of Copper and Zinc in Lead Sulfate

	$PbSO_4$ Calcd.[a]	$PbSO_4$ Found	Difference from Calcd.	Difference from Actual	Element in $PbSO_4$
	Gram	*Gram*	*Mg.*	*Mg.*	%
Pure Pb	0.4106	0.4107	0.1	...	...
	0.4106	0.4107	0.1	...	...
Cu present	0.4106	0.4124	1.8	1.7	0.4
	0.4106	0.4120	1.4	1.3	0.3
	0.4106	0.4121	1.5	1.4	0.3
Pure Pb	0.2875	0.2876	0.1	...	...
	0.2875	0.2878	0.3	...	...
Zn present	0.2875	0.2888	1.3	1.1	0.4
	0.2875	0.2890	1.5	1.3	0.4

[a] Calculated $PbSO_4$ value was determined electrolytically by depositing lead in a known weight of standard lead nitrate solution as PbO_2 on anode.

The data seem to indicate that zinc contamination of lead sulfate could be due to mixed crystal formation, since zinc sulfate and lead sulfate crystallize in the orthorhombic system. It does not seem probable, however, that copper contamination was due to mixed crystal formation, since the pentahydrate of cupric sulfate crystallizes in the triclinic system. It is more likely that adsorption, occlusion, or possibly postprecipitation occurred in these cases.

Conclusions

Iron, manganese, and chromium are coprecipitated with barium sulfate. Cobalt and nickel are not coprecipitated with barium sulfate. The data for iron, manganese, and chromium indicate the formation of complex ions containing these elements.

Copper and zinc are coprecipitated with lead sulfate. The data for zinc indicate the possibility of mixed crystal formation, whereas the data for copper indicate the possibility of adsorption, occlusion, or postprecipitation.

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Extracts from theses submitted by Frederick Rolf and Harold Frediani to the State University of Iowa for the degree of M.S.

Determination of Mercury in Organic Compounds

An Iodometric Procedure Based upon the Method of Rupp

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IN THE iodometric method of Rupp (*20*) mercury is precipitated as metal by reduction of the double iodide with formaldehyde in alkaline solution, the liquid is acidified, and the mercury is dissolved in standard iodine solution, excess of which is finally titrated with thiosulfate. The procedure is less convenient than appears, because long shaking may be required to dissolve the globulated mercury in iodine solution, and because the presence of minute particles of undissolved mercury is hard to detect in the liquid strongly colored with iodine. Results are in general inaccurate: negative errors were reported by Kolthoff and Keijzer (*14*) and by Bruchhausen and Hanzlik (*3*), and persistently high results by Fenimore (*7*) and by Deal (*4*).

Low results due to incomplete reduction of mercury to the metallic state or to incomplete solution of the metal in iodine should be preventable (*21*). In order to facilitate the dissolution of mercury, by precipitating and keeping the metal in finely divided condition, various additions have been suggested—viz., ether and alcohol (*1*), calcium chloride (*2*), and gelatin (*11*), and Kolthoff (*13*) recommended precipitation by hydrogen peroxide added to the solution alkaline with barium hydroxide (*13*). These devices were found to be either only partially effective or not conveniently applicable.

The high results generally obtained by Rupp's method, hitherto unexplained, are probably due to reduction of iodine by formic acid (*5*) produced from formaldehyde by action of alkali and during the reduction of mercuric salt, and present in formalin. Kolthoff and Keijzer (*14*) reported that after heating formalin with alkali the acidified solution absorbed iodine, and attributed this result to formation of "a polymer or a condensation product of formalin". A better interpretation is that formic acid is produced by the Cannizzaro reaction and is the compound which combines iodine. The ability of formic acid to interfere was shown by the series of tests recorded in Table I.

Twenty-five milliliters of 0.1 *N* iodine were pipetted into an iodine flask and 5 ml. of 50 per cent acetic acid added. Formic acid (99 per cent) was introduced in the amount indicated in column 1, and the stoppered flask was allowed to stand in the dark for the period indicated, after which the solution was diluted and the iodine titrated with 0.1 *N* sodium thiosulfate.

Table I shows that formic acid is not immediately oxidized by iodine under the conditions of analysis, but that continued contact leads to appreciable reaction. In Rupp's method this interference would be operative during the more or less prolonged shaking required to dissolve the mercury, and would be the cause of positive error.

TABLE I. REDUCTION OF IODINE BY FORMIC ACID

Formic Acid *Ml.*	Time *Min.*	0.1 *N* $Na_2S_2O_3$ *Ml.*
0 (blank)	45	25.60
1	45	25.20
3	0	25.60
3	45	24.95
3	60	24.80

Unsuccessful attempts were made to replace formaldehyde by a reducing agent such that neither the excess of reagent nor its oxidation product would introduce later complication. The substances tried were acetaldehyde, chloral hydrate, glucose, glycerol (*22*), and ethanolamine (*19*, *23*). As no satisfactory agent was found, it became obvious that the metal must be isolated by filtration. When the liquid is acidified with acetic acid before filtration the mercury globulates to an extent which makes its rate of dissolution in iodine solution prohibitively slow. It had been expected that this difficulty could be overcome by use of bromine (from Koppeschaar's bromide-bromate solution, *15*) instead of iodine, but this was shown not to be the case under the conditions of analysis. It was found, however, that bromine is superior to iodine for the purpose, as its lighter color makes possible the easy detection of traces of undissolved mercury, and because under similar conditions it dissolves mercury more rapidly than does iodine (*24*). Filtration of the precipitated mercury from the still alkaline solution is not a uniformly satisfactory operation, as the metal is in part so finely divided that it creeps badly and may not be wholly retained on the filter. This remaining difficulty is avoided by precipitating with the finely divided mercury some basic magnesium carbonate, presence of which facilitates the operations of decantation and filtration and in no way interferes with completion of the analysis. In this manner the mercury is separated from all substances which might interfere, unless the sample contains some other metal readily reducible to the metallic state, a condition seldom encountered. Removal of mercury by filtration is advantageous also because dissolution of the metal by bromine can be effected in a restricted volume and hence without undue dilution of the bromine.

Comparative trials of several reducing agents, especially formaldehyde and hydrazine (*6*, *12*), led to selection of the latter as the reagent which most uniformly caused rapid and complete separation of mercury in satisfactory physical condition from alkaline solutions of the double iodide.

It was found that an immoderate excess of potassium iodide may partially or wholly prevent reduction of potassium mercuric iodide to mercury in presence of the usual amount of alkali, but that this effect can be counteracted by addition of more alkali.

A solution of 0.1 gram of mercuric chloride in about 50 ml. of water, to which were added 3 ml. of concentrated sulfuric acid and several drops of Koppeschaar's solution, to simulate the initial conditions of analysis, was treated with 15 ml. of 20 per cent potassium iodide solution (instead of the usual 5 ml.). The solution was neutralized with 20 per cent sodium hydroxide and the usual excess of 5 ml. was added. Introduction of 15 ml. of 2 per cent hydrazine sulfate solution caused no precipitation of mercury. Addition of a second 5 ml. of the alkali produced a slight turbidity, and a third 5 ml. of alkali caused a heavy precipitation of mercury. After removal of the metal by filtration no mercury could be detected in the filtrate (sodium stannite test).

In the application of the entire procedure to the analysis of organic compounds, the samples are decomposed by heating with potassium persulfate and concentrated sulfuric acid

(*9*) in an all-glass apparatus with air-cooled condenser. The sulfur dioxide finally present in the mixture may be sufficient to cause separation of mercurous sulfate or metallic mercury upon dilution, to prevent which enough Koppeschaar's solution is added to generate bromine in excess, assuring oxidation of sulfur dioxide and presence of mercury as soluble mercuric salts. The effectiveness of a simple air-reflux (Figure 1) in preventing volatilization losses during decomposition of halogen-containing samples was tested by attaching a water trap to the upper end of the condenser. After normal decompositions of chloromercuriphenol and of diacetoxymercuriphenol mixed with sodium chloride no mercury could be detected in the trap, and the results for mercury were satisfactory.

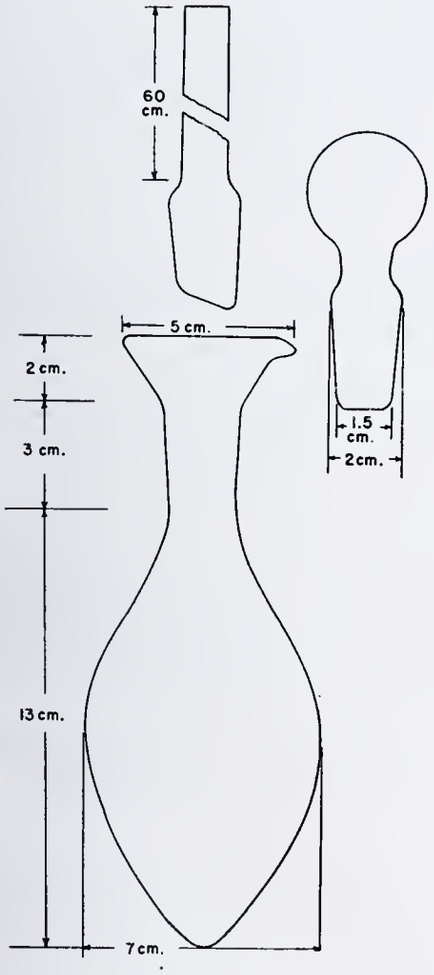

FIGURE 1

Mercury can be precipitated from the alkaline solution of the double bromide (K_2HgBr_4) by formaldehyde, an operation which would permit a small economy with respect to potassium iodide. Tests presented in Table II showed this procedure to be accurate enough for the purpose. It could not be employed in the method developed, because the concentration of salts in the solution (after decomposition by persulfate and sulfuric acid) was sufficient to decrease the stability of the double bromide to such an extent that precipitation of mercuric oxide by alkali could not be prevented by any reasonable excess of potassium bromide.

A 25-ml. aliquot of a solution of mercuric sulfate (from the pure metal), or a weighed sample of purified mercuric chloride in 25 ml. of water, was treated with 10 ml. of 50 per cent potassium bromide solution in an iodine flask. The solution was neutralized with sodium hydroxide, and an excess of 5 ml. of 20 per cent sodium hydroxide solution was added. Mercury was precipitated by addition of 5 ml. of 18 per cent formalin followed by warming. The metal so precipitated was bulky and somewhat granular in appearance. It was removed by filtration and determined by the procedure described below.

TABLE II. PRECIPITATION OF MERCURY FROM ALKALINE SOLUTION OF POTASSIUM MERCURIBROMIDE BY FORMALDEHYDE

Mercury Taken	Mercury Found	Error
Mg.	*Mg.*	*Mg.*
19.7	19.3	−0.4
39.4	39.2	−0.2
39.4	39.2	−0.2
50.5	50.8	+0.3
50.9	50.5	−0.4
89.2	89.3	+0.1
98.8	98.1	−0.7
98.8	98.0	−0.8
98.8	98.4	−0.4
98.8	98.5	−0.3

Apparatus

The 250-ml. flask (Figure 1) is in effect a lemon-shaped iodine flask, with a ground-glass seat for the air-condenser interchangeable with a ground-glass stopper. The flange above the mouth of the flask is provided with a lip for convenience in pouring. It is an advantage to have several flasks for each condenser. In the procedure described the flask serves for decomposition of the sample and for precipitation of the mercury, solution of the metal by bromine, and the final titration.

Procedure

Weigh the sample (about 50 to 100 mg. of mercury) on a piece of cigaret paper or in other convenient manner and transfer to the flask. Introduce 0.5 gram of potassium persulfate, and from a Mohr pipet add 3 ml. of concentrated sulfuric acid in such a way as to wash into the flask any solid particles adherent to the neck. Attach the air-condenser and apply to the tip of the flask the flame of a "micro" burner. Heat gently at first until action subsides, and then heat more vigorously (in presence of chlorine the upper half of the condenser should not become hot; see note *b*, Table III) until organic matter is completely destroyed and the mixture is colorless. This ordinarily requires 20 to 30 minutes. If after heating for some time it becomes obvious that decomposition is slow, allow the mixture to cool somewhat, add through the condenser a suspension of 0.5 gram of potassium persulfate in 1 ml. of concentrated sulfuric acid, and continue the digestion. When decomposition is complete allow the flask and its contents to cool. Through the condenser add Koppeschaar's solution (2.8 grams of potassium bromate and 50 grams of potassium bromide per liter) until excess of bromine persists. Rinse the condenser with water and remove it. If the sample contains iodine some mercuric iodide may sublime into the neck of the flask and the lower part of the condenser. To remove it dip the end of the condenser into a little diluted potassium iodide solution, and pour this into the flask so as to dissolve any mercuric iodide in the neck of the flask. Wash down the surfaces with water and set the condenser aside. The volume at this point should be about 100 ml.

Introduce 5 ml. of 20 per cent potassium iodide solution, or suitably less if some potassium iodide was used previously. Add slowly 20 per cent sodium hydroxide solution until the liquid is alkaline (disappearance of color of iodine) and then 5 ml. in excess. If the liquid becomes turbid owing to separation of mercuric oxide, add 20 per cent potassium iodide solution in 1-ml. portions until a clear solution results. While rotating the flask introduce in a slow stream 10 to 15 ml. of 2 per cent hydrazine sulfate solution, and agitate the mixture occasionally during about 10 minutes. Then add 10 ml. of 5 per cent sodium bicarbonate solution and 5 ml. of 1 per cent magnesium sulfate ($MgSO_4.7H_2O$) solution, both with agitation of the mixture. After about 5 minutes add 5 ml. more of the magnesium sulfate solution, mix well, and allow the flask to stand until the precipitate settles, leaving the supernatant liquid practically clear (10 to 15 minutes).

Decant the solution through a retentive filter paper—e. g., Whatman No. 42, diameter 11 cm.—using a filter cone and light suction. The familiar device of tearing off a corner of the folded paper is not advisable here, as some mercury creeps into the notch. Retain in the flask the greater part of the precipitate, and wash three times with water by decantation. Finally wash the filter three times with water, and transfer the paper and contained precipitate bodily to the flask. Wipe the flange and lip of the flask with a fragment of moistened filter paper, and add this to the contents of the flask.

Table III. Determination of Mercury in Organic Compounds

Compound	Sample	Mercury Found	Mercury Method of White
	Mg.[a]	%	%
Diacetoxymercuriphenol (*27*)	99.95	64.51	64.54
	99.97	64.58	64.73
	100.07	64.54	
	100.10	64.85	
		Av. 64.62	64.64
Diacetoxymercuriphenol with 50 mg. of NaCl	100.02	64.50	
	100.70	64.88	
	100.60	64.64[b]	(64.64)
		Av. 64.67	
Diacetoxymercuriphenol with 50 mg. of KI	99.98	64.84	
	100.02	64.88	(64.64)
		Av. 64.86	
Mercury di-*p*-tolyl, Eastman No. 1448, Hg calcd., 52.42%	100.28	52.16	
	100.13	52.26	
	99.84	52.01	
	100.72	51.97	51.90
	102.45	51.85	52.02
	105.98	51.92	
	118.37	51.98	
		Av. 52.02	51.96
Mercury di-*p*-tolyl with 100 mg. of *o*-iodobenzoic acid	100.33	51.96	
	100.19	52.06	
		Av. 52.01	(51.96)
Chloromercuribenzoic acid, Eastman No. 1858	100.23	51.40	51.80[c]
	100.43	51.50	51.93[c]
		Av. 51.45	51.87[c]
p-Dimethylaminophenylmercuriacetate (*26*), m. p. 165°, Hg calcd., 52.83%	100.82	53.07	
	99.98	53.24	
		Av. 53.16	
o-Chloromercuriphenol (*17*), m. p. 152.5°, Hg calcd., 60.95%	100.84	60.75	
	110.15	61.14	
	100.16	61.25	
		Av. 61.05	
Mercuric cyanide[d], Hg calcd., 79.4%	100.43	79.32	79.35
	99.96	79.08	79.40
		Av. 79.20	79.38
Mercuric *n*-heptyl mercaptide[e], m. p. 74–76°, Hg calcd., 43.34%	110.40	43.39	
	110.10	43.27	
		Av. 43.33	
Mercuric *p*-tolyl mercaptide[f], m. p. 159–161°, Hg calcd., 44.91%	100.30	44.81	
	101.44	44.73	
		Av. 44.77	

[a] Samples weighed on semimicrobalance.

[b] In this analysis a trap containing water was attached to upper end of condenser and decomposition mixture was heated so vigorously that condenser became hot throughout its length. This resulted in appearance of some mercurous chloride in trap. To complete analysis the trap liquid was treated with several drops each of Koppeschaar's solution and sulfuric acid, and combined with decomposition mixture.

[c] Mercury precipitated as sulfide, dissolved in sodium sulfide solution, and reprecipitated by Volhard method. Results probably somewhat high (*9*).

[d] Decomposition effected in open flask (without reflux) to permit escape of hydrogen cyanide, which otherwise appeared to prevent complete reduction of mercury by hydrazine.

[e] Prepared from *n*-heptyl mercaptan (Eastman No. 2122) and mercuric cyanide, both dissolved in alcohol. Recrystallized from alcohol.

[f] Prepared from *p*-thiocresol and purified as above.

Introduce about 25 ml. of water, and then add from a pipet 25 ml. of 0.1 *N* Koppeschaar's solution. While holding the flask quiet add rapidly 5 ml. of 6 *N* sulfuric acid and at once insert the ground-glass stopper. Shake the flask vigorously until the filter paper is completely disintegrated and no dark particles of mercury are visible (about one minute). Immerse the flask in ice water to produce subnormal pressure within, and after a minute or two pour 10 ml. of 20 per cent potassium iodide solution into the gutter of the flask, and loosen the stopper slightly to allow the potassium iodide solution to pass into the flask. Shake the tightly stoppered flask to absorb in the liquid the vapors of bromine, then remove the stopper and rinse it and the mouth of the flask with water.

Titrate the iodine with 0.05 *N* sodium thiosulfate solution standardized against potassium iodate (*16*). When the iodine color is faint stopper the flask and shake well, to dislodge traces of iodine adherent to the paper. Then add 3 ml. of 0.5 per cent solution of soluble starch and complete the titration. Determine the effective strength of the bromide-bromate solution in a blank titration conducted exactly as described in the preceding paragraph, a filter paper being introduced to compensate any effect due to the filter paper present in the analysis (about 0.07 cc. of 0.05 *N* thiosulfate). Calculate the mercury present from the difference between the two titrations (1 ml. of 0.05 *N* thiosulfate represents 0.005015 gram of mercury).

Analytical Results

The accuracy of the procedure described in the last four paragraphs—i. e., exclusive of the decomposition of organic material—was tested by determinations of mercury in mercuric chloride purified by two recrystallizations from water. Using samples of about 0.1 gram the results were 73.83, 73.74, 73.82, and 73.72 per cent, average 73.78 per cent. The calculated value is 73.88 per cent.

Results obtained in the analyses of organic mercury compounds are presented in Table III. As some of the compounds examined contained other than the theoretical amounts of mercury, and in many cases the purity of organic mercury compounds may not be ascertainable except by analysis, the accuracy of the new procedure was tested by parallel analyses using an umpire method. The method of White (*25*) served for compounds containing no halogen, the mercury being titrated with potassium thiocyanate solution standardized against pure silver. The presence of halogen increases the likelihood of loss of mercury during the White decomposition, and excludes the thiocyanate titration, making necessary the determination of mercury as mercuric sulfide, a procedure affected in this case by relatively large positive errors (*8*, *18*). That the method described is applicable to halogen-containing compounds was shown by the analysis of halogen-free organic compounds in presence of added sodium chloride or potassium iodide. It is an advantage of Rupp's method, and therefore of the new procedure, that the presence of halogen, and especially of iodine (*10*), introduces no obstruction. The method described was tested finally by independent trials made by an analyst previously unfamiliar with it. The two sets of results, included in Table III, show no important differences.

The analysis requires about 3 hours for a single sample. If several decomposition flasks are available, a like number of analyses may be completed in little more time. The method is believed to be widely applicable, and it yields results considerably better than those obtainable by the Rupp procedure. The accuracy of the results appears to be satisfactory, and the variations among duplicates are not greater than is to be expected of a volumetric method in view of the high equivalent weight of mercury. It seems probable that good results need not be attributed to compensatory errors, but to absence of irregularities in the individual operations of the analysis.

Work is now in progress on an analogous method for the determination of arsenic in organic compounds.

Summary

The high results obtained by Rupp's iodometric method for determination of mercury are probably due to reduction of some iodine by formic acid produced from formaldehyde during precipitation of mercury or by Cannizzaro reaction in the initially alkaline liquid. Reducing agents other than formaldehyde also interfere in some way with completion of the analysis by the Rupp procedure. It is therefore necessary to remove mercury by filtration before it is determined iodometrically.

Mercury can be precipitated similarly from alkaline solution of the double bromide, but in presence of dissolved salts in quantity (following decomposition of organic samples) the double bromide becomes too unstable to prevent precipitation of mercuric oxide upon addition of alkali.

Mercury is precipitated in such physical condition as to be both filterable and rapidly dissolved by action of bromine by addition of hydrazine sulfate to the alkaline solution of the double iodide, and it is kept in this condition during the operations of filtration and washing by presence of some precipitated basic magnesium carbonate.

Bromine (from Koppeschaar's bromide-bromate solution) is preferable to iodine for re-solution of the separated mercury, as it acts more rapidly and avoids the visual difficulty in detecting minute particles of undissolved metal in the liquid colored by excess iodine.

Based on Rupp's original method and the findings mentioned above, a new procedure for determination of mercury in organic compounds was elaborated. The organic sample is decomposed by means of persulfate and sulfuric acid. The procedure is convenient, moderately rapid, and applicable in presence of halogens.

Acknowledgment

Grateful acknowledgment is made to Martha Torrey and Charles Judson, who prepared and purified several of the mercury compounds analyzed, and to Ezra Staple, who executed several series of confirmatory analyses.

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THIS paper represents a completed portion of the experimental study to be submitted in partial satisfaction of the requirements for the Ph.D. degree at the University of Pennsylvania.

Photometric Method for Estimation of Minute Amounts of Mercury

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IT HAS been shown by a number of investigators (*4*, *5*, *7*) that mercury resonance radiation of wave length 2537 Å. is absorbed by mercury vapor. If a constant source of this radiation (General Electric 4-watt germicidal lamp) is placed at one end of a tube and a narrow-band photoelectric cell sensitive to this line is placed at the other end, a microammeter, connected through an amplifying system to the photocell, will indicate the amount of light falling on the cell. It is evident that, if the tube contains mercury vapor, some of the light will be absorbed and the microammeter will give a different reading from that given when no mercury vapor is in the light path.

This is the principle of the mercury-vapor detector developed by Woodson (*6*) to determine the amount of mercury vapor in air. Hanson (*2*) has shown that a number of organic solvent vapors absorb this radiation (although to a much lesser degree than an equal weight of mercury vapor), and his Tri-Per-Analyzer is based on the same principle.

These instruments have been designed to estimate mercury or solvent vapors in large volumes of circulating air, and are not applicable to the estimation of minute amounts of mercury present in solution.

The authors have observed that, on heating mercuric sulfide in a closed system in a quartz-ended cell (Figure 1), the amount of absorption of the 2537 Å. radiation is constant and reproducible for a given amount of mercuric sulfide.

It is obvious that if a simple means were available for converting mercuric ions to the sulfide and collecting the mercuric sulfide formed, the photometric method could be used for the determination of mercuric compounds in solution.

Clarke and Hermance (*1*) have shown that minute amounts of metals (the sulfides of which are less soluble than cadmium sulfide) are completely removed by allowing solutions of the metal ions to filter slowly through filter paper impregnated with cadmium sulfide. Some modification of this recovery method is required, since filter paper or other organic matter would be decomposed on subsequent heating, with the formation of smoke, and this would seriously affect the photometric method for the estimation of mercury. The authors have observed that a pad of preignited asbestos fibers impregnated with cadmium sulfide is equally efficient in removing mercury ions from solution and the mercury sulfide so obtained is in an ideal condition for the subsequent thermal treatment. These adaptations of well-known principles are the basis of the method presented.

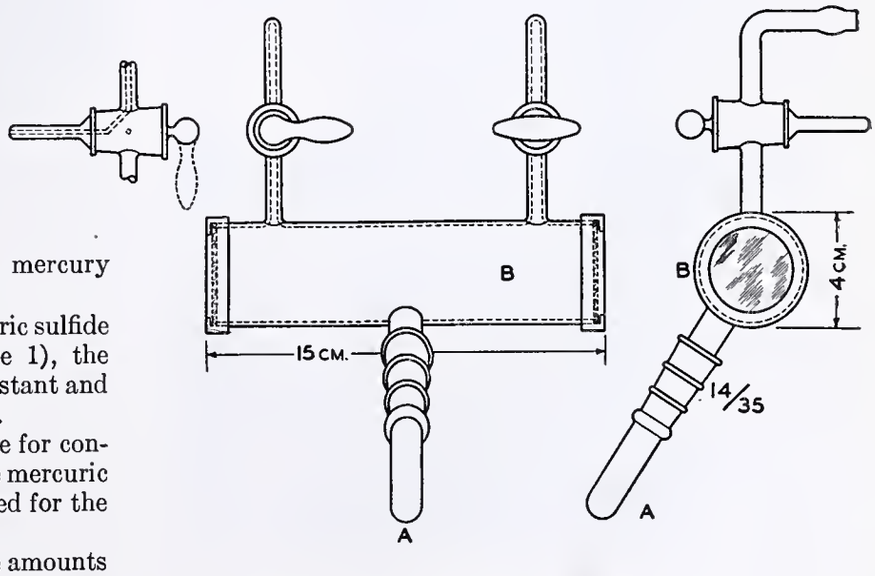

FIGURE 1. ABSORPTION CELL

Recovery of Mercury

REAGENTS. *Cadmium Sulfide–Impregnated Asbestos Fiber.* Acid-washed and ignited long-fiber asbestos is cut into pieces approximately 1.25 cm. (0.5 inch) long and fluffed up in a stream of air (*3*). Five grams of the well-fluffed material (generally freed from any hard pieces) are placed in 100 cc. of 15 per cent cadmium acetate solution and allowed to soak for 5 minutes. The asbestos is drained off through a coarse sintered-glass funnel. The pad, weighing approximately 45 grams, is peeled from the sintered-glass disk, placed in 100 cc. of 5 per cent sodium sulfide solution, and stirred gently for 5 minutes. The impregnated asbestos fibers are separated from the solution again on the coarse sintered-glass funnel and most of the excess sodium sulfide is removed by washing directly on the funnel with distilled water, using suction. The slightly moist pad is peeled off and stirred in 100 cc. of 5 per cent cadmium acetate solution for 5 minutes. The fibers are washed as before, but much more thoroughly, the moist asbestos pad being removed from the filter and dispersed by stirring in 2 liters of water and refiltering. This procedure is repeated 8 to 10 times before the final sucking down to dryness on the filter funnel.

The pad is removed, dried in the oven at 105°, and ignited in a muffle furnace at 550° for 1 hour. After cooling, one half of the sample is placed in a 2000-cc. glass-stoppered flask, 1000 cc. of mercury-free water are placed in the flask, and the flask is well shaken to disperse the fibers. A certain amount of the asbestos will settle to the bottom of the flask, as it was not possible to fluff it enough to give complete dispersion. This should be disregarded and the suspension should be only gently swirled before taking a sample for the final dilution, in which 100 cc. of the above suspension are diluted to approximately 450 cc. with mercury-free water.

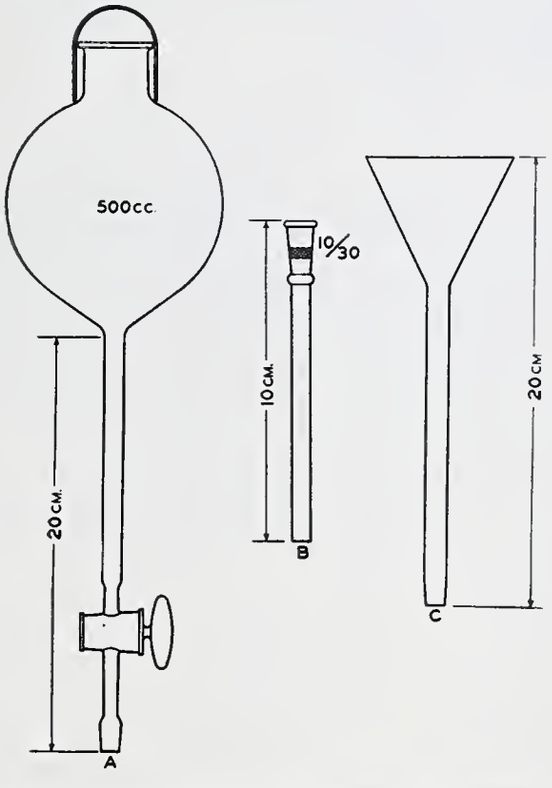

FIGURE 2. APPARATUS FOR RECOVERY OF MERCURY

Mercury-Free Water. This is prepared by stirring 25 cc. of the cadmium sulfide–impregnated asbestos fiber suspension with approximately 2 liters of distilled water and, after allowing to stand a few minutes, filtering the solution through a sintered-glass funnel.

Mercury-Free 80 Per Cent Methyl Alcohol. Eight hundred cubic centimeters of methyl alcohol and 200 cc. of distilled water are treated with 10 cc. of the cadmium sulfide–impregnated asbestos fiber suspension and, after standing a few minutes, the solution is filtered through a clean sintered-glass funnel.

APPARATUS. The apparatus required (Figure 2) is a simple long-stemmed flask, *A* (having at its end a shortened $\frac{10}{30}$ joint), to hold the sample solution, and tube *B* to hold the cadmium sulfide–impregnated pad. (Two short V-shaped slots are made with a triangular file across the bore of the stopcock plug in flask *A*, along the circumference of rotation extending equidistant on either side of the bore. This facilitates regulation of flow.) The pad is prepared in holder *B* by means of the funnel, *C*.

FIGURE 3. BATTERY OF EXTRACTORS

The asbestos pad is held in *B* by means of a piece of coarse sintered glass, approximately 3 mm. thick, ground to fit about half way down the standard taper joint. This is easily prepared by cementing a piece of sintered glass (ground to approximately the right size on a slow-speed wet emery wheel) to a piece of glass tubing with de Khotinsky cement. It is then finally ground into the standard taper joint manually, using a slurry of medium fine emery as abrasive.

PROCEDURE. A sintered-glass disk is introduced into tube *B* by running a glass tube up from the bottom of the tube, placing the glass disk on it, and gently withdrawing the tube; the disk will readily seat itself. The tube is then connected to funnel *C*, and 25 cc. of the well-shaken asbestos suspension are poured into the funnel and allowed to settle slowly onto the disk. The tube is disconnected from the funnel and the pad is drawn down on the glass disk by means of suction applied at the lower end of the tube.

If the pad is not uniformly distributed over the surface of the disk, it is wetted with mercury-free water from an all-glass wash bottle and again drawn down on the disk. This procedure is repeated until the pad appears to be uniformly distributed. Twenty-five cubic centimeters of mercury-free water are placed in *A* (Figure 2) and the stopcock is opened slightly to fill the tube to the bottom of the ground joint, in order to forestall the possibility of bubble formation, either in the stopcock or directly above the asbestos pad.

Tube *B* containing a freshly prepared pad is filled with water above the asbestos pad by holding it under *A* and carefully opening the stopcock; it is then quickly connected to *A* by means of the standard taper joint. The solution under investigation (150 to 400 cc.) is adjusted to pH 5 to 7 and immediately placed in the flask, and the stopcock is turned so that the sample passes through the cadmium sulfide pad at about 2 to 4 cc. per minute. After the solution has passed through the pad, *B* is disconnected from the flask, washed with mercury-free water, and finally rinsed a few times with 80 per cent methyl alcohol (mercury-free) from a glass-stoppered wash bottle. The pad is finally sucked down to a compact pad on the sintered-glass disk, and air is drawn through it for a few minutes by means of a water suction

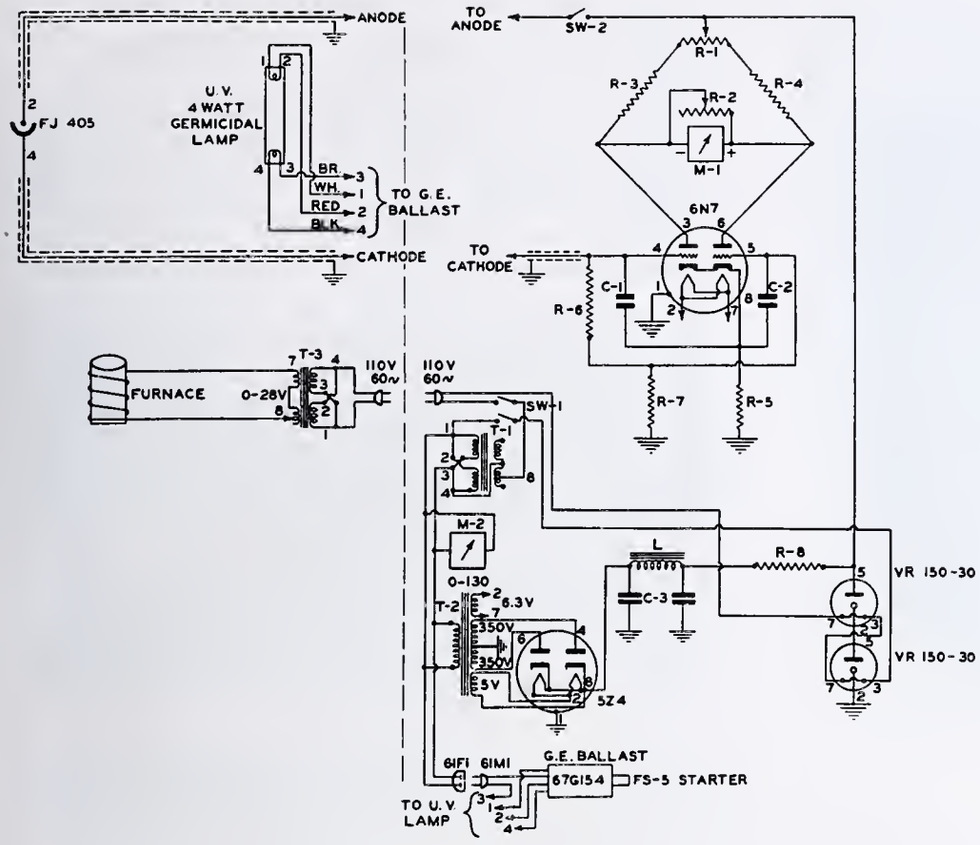

FIGURE 4. SCHEMATIC DIAGRAM OF CIRCUIT

C-1. 0.002 mfd.
C-2. 0.002 mfd.
C-3. 8–8 mfd.
FJ-405. Phototube
L. 15-henry choke
R-1. Bridge balance, 3000 ω.
R-2. Sensitivity control, 1000 ω.
R-3. 1000 ω.
R-4. 1000 ω.
R-5. 15,000 ω.
R-6. 5,000,000 ω.
R-7. 500,000 ω.
R-8. 5000 ω.
M-1. 0–300 D. C. microammeter
M-2. 0–130 A. C. voltmeter
SW-1. D. P. S. T. on-off power supply switch
SW-2. S. P. S. T. bridge balancing and phototube disconnecting switch
T-1. VL-0 Universal Varitran
T-2. Power transformer
T-3. VL-2 Universal Varitran

pump. A glass rod is introduced from the bottom of the tube and the glass disk is gently forced up to the level of the top of the standard taper joint. The asbestos pad is peeled off from the glass disk, using a pair of steel tweezers, and placed in the depression of a spot plate in a vacuum desiccator and the desiccator is evacuated by means of a water pump for approximately 1 hour. The pad is now ready for determination as directed below.

NOTES ON RECOVERY. The cadmium sulfide–asbestos pad should contain no very thin spots through which the solution could pass without completely removing all the mercury present; a simple visual observation will suffice to detect any thin spots. The dried pads weigh approximately 10 mg. Through properly prepared pads the rate of flow of solution has been increased to approximately 10 cc. per minute with complete recovery of mercury. But, using a battery of eight extractors (Figure 3), there is no advantage in speeding up the rate because the pads must be prepared with much greater care and the routine can be so organized that no time is lost at the rate of 4 cc. per minute.

The efficiency of the pads in recovering mercury from solutions should be tested by allowing 0.5 microgram of mercury as nitrate in 200 cc. of solution to flow through a pad at the rate selected. The solution is collected in a flask and again passed through a new pad at the same rate. The pads are dried and the mercury is determined in each pad by the method given below. The second pad should not contain over 0.01 microgram of mercury (corrected for blank or untreated pad) as calculated from the calibration curve.

A blank pad, when tested as directed below, should not give a deflection on the microammeter greater than that given by 0.02 microgram of mercury.

The dried pads containing mercury are stable and a number of them may be prepared, using a battery of recovery units and the mercury determined later in a group, or for routine determinations the recovery and determination of groups can be alternated, so that the operations are carried on simultaneously.

If the solution contains suspended material, this must be removed by filtering through a clean sintered-glass filter or a filter paper that has been washed with 1 to 4 nitric acid solution.

Mercuric ions are adsorbed to glass from dilute solutions. The solutions used for calibration and those under investigation should, therefore, be run as soon as prepared. All solutions should be prepared in Pyrex glass.

Tube *B* (Figure 2) containing the sintered-glass disk is rinsed with strong nitric acid, washed, and finally rinsed with mercury-free water just before use.

Determination of Recovered Mercury

APPARATUS. Figure 4 is a schematic diagram of the circuit. Figure 1 is a front and side view of the absorption cell, a 4-cm. Pyrex tube ground and polished on the ends, to which two stopcocks and a standard taper male joint are sealed. The stopcocks allow the cell to be swept out with nitrogen and the standard taper joint is used to attach the pad-holding tube, which is heated to evolve mercury vapor. To facilitate cleaning the cell, detachable quartz ends are employed. Male threaded collars are cemented over the outside of the 4-cm. tube, which projects 1 to 2 mm. through the collars. Quartz disks 2 mm. thick are held tightly against the polished glass ends by means of metal screw caps, fitting the male collars. The screw caps are fitted with rubber gaskets similar to those used in polariscope tubes.

Figure 5 (coded to agree with Figure 4) shows the arrangement of essential electric circuit controls and reading dial. Figure 6

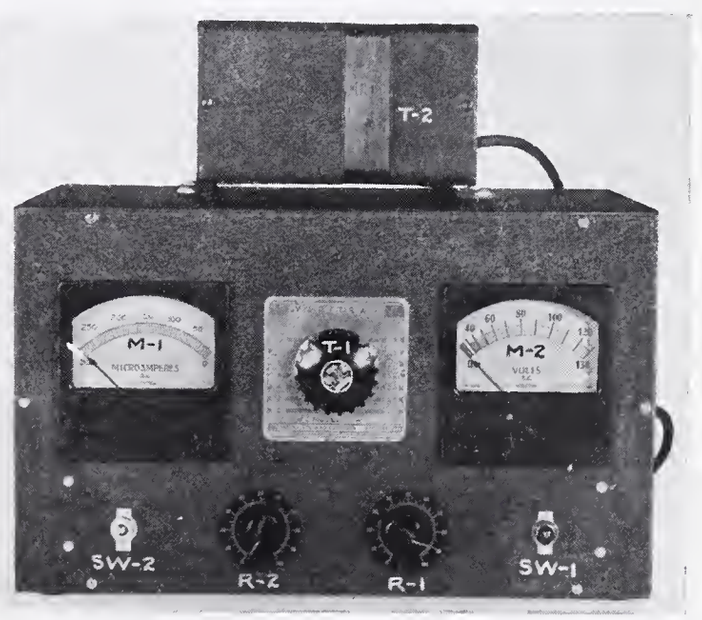

FIGURE 5. ELECTRIC CIRCUIT CONTROLS AND READING DIAL

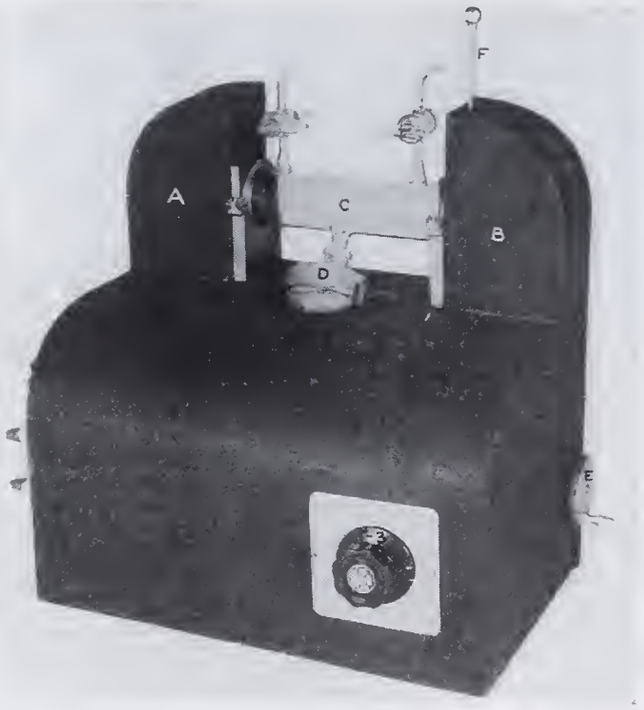

FIGURE 6. EVOLUTION AND ABSORPTION UNIT

shows the arrangement of parts of the evolution and absorption unit. The metal cell holder and the photocell are thermally insulated from other metal parts of the unit, which, although well vented in the rear and bottom, become warm by radiation from the tube furnace. This is made by winding 155 cm. of 22BS 0.0643-cm. (0.0253-inch) Nichrome wire on a 30-mm. diameter 80-mm. high Alundum extraction thimble, and covering the windings and bottom with asbestos cloth. The inner furnace temperature was measured with a thermocouple for various settings of the Varitran and the observed voltages necessary to maintain temperatures of 450° and 550° C., respectively, were used in subsequent mercury determinations. When a series of diaphragms was used to collimate the beam falling on the photocell, after passing through the absorption cell, the amplified electrical response was unsatisfactory because the light energy falling on the photocell was too low. The lamp and photocell were, therefore, mounted close to the quartz ends of the absorption cell to take advantage of reflections from the inner glass wall. The cell was held in a fixed reproducible position.

PROCEDURE. The two operating switches of the instrument are turned on (*SW*-2, Figure 5, the electrical bridge-balancing switch, and *SW*-1, Figure 5, the control switch for the quartz low-pressure mercury-vapor source) and the furnace Varitran (*T*-3, Figure 6) is plugged into a 110-volt alternating current line The Varitran is then set to the predetermined voltage necessary to maintain the tube furnace at about 550° C. The voltage regulator (*T*-2, Figure 5), which supplies a constant voltage to the electrical circuit and light source, is permanently plugged into a 110-volt alternating current line in order to minimize the warm-up period. About 15 minutes after the switches have been turned on, the instrument may be tentatively adjusted to the initial settings as outlined below.

The Varitran controlling the source voltage (*T*-1, Figure 5) is adjusted to deliver 110 volts, as read on the voltmeter (*M*-2, Figure 5). The bridge-balancing adjustment switch (*SW*-2, Figure 5) is turned off and the bridge-balance dial (*R*-1, Figure 5) is turned until the microammeter gives a reading of exactly 300 divisions. The bridge-balance switch (*SW*-2, Figure 5) is then turned on, and if no light is falling on the photocell, the microammeter will maintain the same reading. The sampling tube (*C*, Figure 6) is swept out with nitrogen for a minute to remove any residual vapors, and the shutter (*F*, Figure 6) is then opened. The microammeter shunt adjustment knob (sensitivity control, *R*-2, Figure 5) is adjusted until the meter reads exactly zero. The shutter is then closed.

This routine of tentative adjustment is followed every 10 to 15 minutes until the change in microammeter readings is less than 5 divisions per 15-minute interval. The instrument as finally adjusted should give a microammeter reading of 300 divisions when the shutter is closed, and zero when the shutter is open. A sample pad is placed in the evolution tube (*A*, Figure 1), nitrogen

TABLE I. REPRODUCIBILITY OF READINGS

Micrograms of Mercury	Readings, Corrected for Blank Pad							Average
0.10	39	36	34	37	34	36	36	36
0.20	76	74	68	70	...	...	..	72
0.30	91	93	91	94	95	92	..	93
0.40	113	111	118	114	...	...	..	114
0.50	135	135	143	139	138	133	..	137
0.70	158	159	149	157	156	...	..	156

is passed through the absorption cell for 2 to 3 minutes, and the stopcock is closed. The tube furnace (*D*, Figure 6) is raised to enclose the evolution tube, by means of handle *E* (Figure 6).

The furnace Varitran is now readjusted to the predetermined voltage necessary to maintain 450° C., and a timing clock is started. The deflection of the microammeter is read after 4 minutes with the shutter open only long enough to make the reading. Successive readings are made at 2-minute intervals; when the deflection changes only 2 to 3 divisions from the previous reading, a final reading is taken. (This normally occurs 6 to 10 minutes after beginning the heating of the pad.)

It is necessary to construct a calibration curve for converting the microammeter readings to the mass of mercury. This is prepared by allowing known amounts of mercuric nitrate contained in 200 cc. of solution to be recovered as above. The mercury is evolved from the pads as outlined and the readings on the microammeter are observed.

Table I shows the reproducibility of readings. Figure 7 was plotted from the data given in Table I and was used for converting microammeter deflections given by unknowns to mass of mercury.

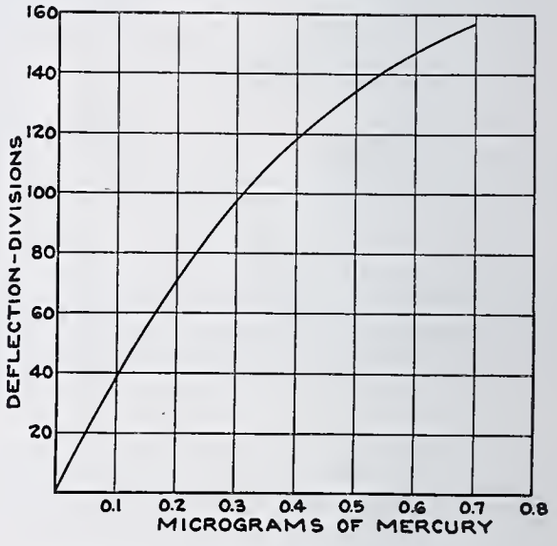

FIGURE 7. DETERMINATION OF MERCURY

For subsequent determinations the furnace is run down, and the Varitran controlling its voltage is altered to the voltage necessary to hold the temperature at 550° C. The mercury vapor is swept out for a few minutes with nitrogen, or until on opening the shutter the instrument again gives a scale sweep of 300 units, showing that the cell has been freed of mercury vapor. Tube *A* is detached at the standard taper joint, a tube containing another sample pad is connected, and, just prior to raising the furnace to heat the pad, the scale sweep of the microammeter is checked.

If necessary, the instrument is adjusted so that a full-scale sweep of 300 divisions is obtained when the shutter is opened. After the instrument has been brought to temperature equilibrium (approximately 1 hour), the instrument is fairly stable and necessary readjustments are slight.

NOTES ON DETERMINATION. If the shutter is kept open and light is allowed to pass continually through the cell, the reading on the microammeter will slowly sink to zero, even when mercury vapor was present in the cell before opening the

shutter. This is not observed, or at least is not of the same time order, when the shutter is kept closed and only opened for a few seconds while readings are being made. Whether or not this effect is caused by mercury being deposited on the walls of the glass cell under the influence of the light has not yet been ascertained. (Calculations show that the amount of mercury required to saturate the nitrogen in the cell is much larger than the amounts present.) If the cell is filled with vapor containing mercury and the light source allowed to pass through until a zero reading is obtained, the shutter closed, and the cell allowed to stand, a partial regain of the absorption effect will be observed. If carried over to a subsequent determination, this in effect will cause erroneous readings, and a probable explanation is that the ionized mercury vapor is more readily adsorbed to the glass surface than the unionized vapor. In accordance with the above observation, the authors have found it necessary to keep the shutter closed except for the shortest time required to make a deflection reading.

The apparatus should not be operated in a room illuminated by daylight, which is variable, unless the evolution unit (Figure 6) is shielded from the light. An inside room lighted artificially has been found satisfactory.

Summary

The method presented allows the determination of 0.02 to 0.60 ± 0.02 microgram of mercury in 150 to 400 cc. of solution. Using a battery of recovery units as shown, an operator in these laboratories has been able to make 25 determinations per day. The instrument described has been in operation for 6 months without noticeable change in operating characteristics.

The method has been used with water-miscible solvent mixtures and with solutions from the digestion of solid organic materials. It would appear to be applicable to the estimation of mercury in biological materials after suitable preliminary digestive treatment.

Acknowledgments

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Separation of Bismuth from Lead with Ammonium Formate

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The sodium formate method, proposed by Benkert and Smith for the quantitative separation of bismuth from lead, shows definite advantages over other procedures, but there are obstacles to its general adoption.

A new procedure is proposed, suited for both the quantitative separation and determination of bismuth and lead and for the quantitative separation of small amounts of bismuth from large amounts of lead.

The nitric acid solution of bismuth and lead is neutralized with ammonia and ammonium carbonate. Ammonium formate is added and the precipitate of basic bismuth formate is filtered off, washed with hot water, reprecipitated, and finally ignited to the oxide. The precipitate can also be dissolved in hydrochloric acid and bismuth determined as oxychloride. If the bismuth formate precipitate is very small it can be dissolved in dilute sulfuric acid and bismuth determined colorimetrically with potassium iodide.

Lead is precipitated in the filtrate of the bismuth formate as chromate with potassium or ammonium dichromate.

MANY laboratories engaged in industrial or research analysis find it necessary to carry out numerous quantitative separations of bismuth from lead, and many cases arise where the time required to perform such a procedure must be kept at a minimum. A comprehensive survey of the literature shows that a large amount of work has been done on methods for the quantitative separation of bismuth from lead, but in general the procedures have not been entirely satisfactory.

The investigation here reported was undertaken with a view to developing a method which would be simple and easy of manipulation and at the same time give results comparable with those obtained by the more complicated methods—in other words, a strictly routine method that could be really depended upon.

Existing Methods

Methods given by standard texts for quantitative separation of bismuth from lead are, with few exceptions, based upon the fact that in weak nitric acid solution the bismuth ion is decomposed upon the addition of water or salts of weak acids, forming basic bismuth compounds.

Probably the best known of these methods was suggested by Loewe (*13*). Briefly, it consists in precipitating bismuth as

basic nitrate of indefinite composition, by evaporating the nitric acid solution to sirupy consistency, adding water, evaporating to dryness, and repeating this operation until further addition of water fails to produce a turbid solution. Lead is finally extracted with a cold 0.2 per cent solution of ammonium nitrate. This method is generally considered very reliable, although Hillebrand and Lundell (*7*) and Herzog (*6*) state that the separation is not entirely satisfactory. Its chief weakness lies in the fact that its application is confined to the separation of bismuth from moderate amounts of lead. Furthermore, chlorides and sulfates, which also form basic compounds with bismuth and cause the retention of some lead, must be absent. The method is time-consuming because of the repeated evaporations.

Various modifications of this method have been proposed since its early use. Luff (*16*) reduces the hydrogen-ion concentration of the solution by adding sodium nitrite to the weak nitric acid solution of bismuth and lead which has previously been neutralized with sodium bicarbonate. According to its author, this method is limited to amounts of bismuth and lead not exceeding 100 mg. Ammonium salts must be absent. The method is not accurate because of the retention of alkali by the precipitate and is not attractive because of the necessity for more or less tedious purification procedures.

Blumenthal (*2*) suggests neutralizing the excess nitric acid with a freshly prepared emulsion of mercuric oxide, and states that as much as 50 mg. of bismuth can thus be separated from 10 grams of lead in one operation. The method is not suited to a simultaneous determination of the lead and bismuth content. The mercuric oxide–bismuth subnitrate precipitate must stand for at least 12 hours to guarantee complete precipitation of the bismuth. The procedure makes it necessary to precipitate and determine bismuth finally as the phosphate, a method that, according to Hillebrand and Lundell (*9*), should not be considered in accurate analyses. In addition, Blumenthal's method is subject to many interfering elements and is hence not practical for commercial analysis. The present writer proposed in a previous paper the use of zinc oxide, in a similar procedure (*12*). One zinc oxide precipitation does not yield a quantitative separation of bismuth from lead, but permits precipitation as bismuth oxychloride.

A separation of bismuth from lead, based on precipitation of the bismuth as basic acetate, is suggested by Herzog (*6*). The method is very time-consuming and has obviously not given satisfactory results in hands other than those of its author (*1*). The separation of bismuth from lead by precipitating the lead as lead sulfate, first suggested by Fresenius (*3*) and recommended by such trustworthy investigators as Scott (*19*) and the Gesellschaft Deutscher Metallhütten- und Bergleute (*5*) is worthless, although often used by many analysts. The lead sulfate, whether thrown out from dilute nitric acid solution by adding an excess of sulfuric acid, or obtained by fuming with sulfuric acid the nitric acid solution of bismuth and lead, invariably occludes bismuth, as stated by Hillebrand and Lundell (*8*), Herzog (*6*), Hills (*10*), Blumenthal (*2*), and Low (*14*). The precipitation of bismuth as bismuth oxychloride, first advocated by Rose (*18*), does not yield a quantitative separation of bismuth from lead, particularly when large amounts of the latter are involved, two reprecipitations being necessary to guarantee complete separation of the two metals. However, a procedure for the preliminary separation of small amounts of bismuth (5 to 50 mg.) from large amounts of lead, as recommended in the well-known Ledoux method (*15*), is based on precipitation of bismuth as bismuth oxychloride. The method is rapid and accurate under certain conditions, but variations in conditions may produce erratic and irregular results. Considerable difficulties are also encountered if only minute quantities of bismuth are involved (*11*).

The only other method which needs to be considered here calls for precipitation of bismuth as oxybromide (*17*), as proposed by Moser and Maxymovicz. It is on a par with the oxychloride method, though not so widely used. Its authors state that the oxybromide furnishes a better separation from lead than does precipitation as oxychloride or basic nitrate. Ammonium salts and chlorides, however, should be absent and not more than 0.35 gram of lead must be present. The method calls for reprecipitation of the bismuth oxybromide precipitate, bismuth finally being determined as phosphate because of the volatility of the oxybromide. This method is not suited for a simultaneous determination of lead and bismuth.

Other methods for the separation of bismuth from lead add nothing of value to those mentioned, although some, such as precipitation of bismuth by means of cinchonine hydrochloride (*4*), are capable of giving good results in special cases.

Of all the methods mentioned, only the one suggested by Loewe (*13*) is suited for the quantitative separation and determination of the two metals. All other methods depend largely on the ratio of the amounts of bismuth and lead and are suited only for the quantitative determination of bismuth.

Table I. Accuracy of Benkert and Smith's Method

Bismuth Used	Bismuth Found				
	In precipitate	In Filtrates			
		I	II	III	IV
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
10.0	9.1	0.3	0.2	0.3	0.1
20.0	18.6	0.4	0.2	0.5	0.1
50.0	48.3	0.6	0.2	0.6	0.1
50.0	48.5	0.6	0.1	0.5	0.1
75.0	73.2	0.8	0.2	0.7	0.2
100.0	97.5	1.0	0.3	0.9	0.2
100.0	97.1	0.9	0.3	1.3	0.3
200.0	197.0	1.1	0.4	1.3	0.3
300.0	296.8	1.4	0.3	1.2	0.3

Sodium Formate Method

A method which has scarcely been mentioned in the literature was proposed by Benkert and Smith (*1*) who, some 50 years ago, in calling attention to the defects in Herzog's procedure (*6*), reported that they were able to separate bismuth and lead by means of sodium formate, an idea closely related to that of Herzog.

The nitric acid solution of bismuth and lead is "almost neutralized with sodium carbonate, or until the incipient precipitate dissolves slowly, when considerable sodium formate solution of specific gravity 1.084 and a few drops of aqueous formic acid are added". The solution is heated to boiling and held at this point for 5 minutes. The precipitate is allowed to settle and filtered while hot, then dissolved in nitric acid and precipitated with ammonium carbonate. The basic bismuth carbonate contaminated by some lead carbonate is dissolved in nitric acid and the formate precipitation is repeated. The resulting precipitate, which is contaminated by sodium salts, is again dissolved in nitric acid and bismuth is finally precipitated with ammonium carbonate and ignited to the oxide.

The experimental data submitted by Benkert and Smith are extremely meager, and in the investigation here reported the method was tested as regards completeness of precipitation and separation with a view toward possible simplification and improvement. A new procedure incorporating the best features of Benkert and Smith's method has been developed which, in the writer's opinion, has definite advantages over the methods in use at present.

To test the completeness of the bismuth precipitation, varying amounts of bismuth were dissolved in nitric acid and the precipitation of basic formate was carried out, as called for by the authors. The precipitate was filtered off and washed with hot water, retaining the filtrate (I). The precipitate was dissolved in nitric acid and bismuth precipitated as basic carbonate. The filtrate of the basic bismuth carbonate was also retained (II). The precipitate was redissolved in nitric acid and the basic formate and basic carbonate precipitations were repeated, yielding two more filtrates (III, formate, and IV, carbonate). The final basic bismuth carbonate precipitate was ignited to and weighed as bismuth oxide, Bi_2O_3. In addition, the four filtrates were colorimetrically tested for bismuth (Table I).

Table I shows conclusively that bismuth subcarbonate is somewhat soluble in an excess of ammonium carbonate. The precipitation of bismuth as subcarbonate should therefore be avoided, whenever possible. The data also indicate the obstacles to a general adoption of Benkert and Smith's method—namely, the incomplete precipitation of bismuth as basic formate under the conditions called for.

Endeavoring to ascertain why such low results are obtained with this method, comprehensive tests were carried

out and it became apparent that the primary cause of the discrepancies is lack of information concerning the exact acidity at which the precipitation of basic bismuth formate should be carried out. The authors merely state that the nitric acid solution of bismuth and lead should be "almost neutralized with sodium carbonate or until the incipient precipitate dissolved slowly". That these data are too vague and inexact is shown by the following illustration:

Portions of 25 mg. (I) and 200 mg. (II) were dissolved in 8 *N* nitric acid and the solution was carefully neutralized with a 10 per cent (1.89 *N*) solution of sodium carbonate, until the addition of one more drop of sodium carbonate would have caused precipitation of bismuth carbonate. Methyl orange was added at this point, indicating a hydrogen-ion concentration lower than that equivalent to pH 3 (red color). The neutralization with sodium carbonate was continued until the yellow color developed, indicating a hydrogen-ion concentration greater than that equivalent to pH 4.5, using in I 1.4 ml. and in II 1.8 ml. Then 8 *N* nitric acid was added until the precipitate which had formed during the neutralization procedure just dissolved, using in I 0.8 ml. and in II 1.3 ml. The volume of the solution was in I 150 ml. and in II 200 ml. As 1 ml. of 8 *N* nitric acid neutralizes 4.3 ml. of 10 per cent sodium carbonate, the excess nitric acid required to keep the bismuth in solution can thus be calculated.

$$\text{Excess } HNO_3 = 0.8 - \frac{1.4}{4.3} = 0.47 \text{ ml.}$$

$$\text{Excess } HNO_3 = 1.3 - \frac{1.8}{4.3} = 0.88 \text{ ml.}$$

Accordingly the acidity in I amounted to 0.025 *N* and in II to 0.035 *N*.

The foregoing data would indicate that Benkert and Smith by precipitating bismuth formate from a clear nitric acid solution, neutralized with sodium carbonate, carried out the precipitation at an initial acidity of between 0.02 and 0.04 *N* (pH 1.7 to 1.4). It is obvious that the excess nitric acid will react with sodium formate, causing the formation of formic acid. The concentration of the latter is, as suggested by the authors, even increased by a further addition, thus making it necessary to add "considerable" amounts of sodium formate as buffer. But even this precaution does not yield a complete precipitation of bismuth (as shown in Table I), probably because of the formation of complex bismuth formate.

To determine the accurate permissible acidity range, 200-mg. portions of bismuth were dissolved in nitric acid. The solution was neutralized with 10 per cent sodium carbonate, using methyl orange as indicator, until the addition of one more drop of sodium carbonate would have produced the yellow color of methyl orange, indicating a hydrogen-ion concentration equivalent to about pH 4 (at this stage a part of the bismuth had already separated). The solution was acidified with varying amounts of 8 *N* nitric acid, and 40 ml. of 10 per cent sodium formate solution were added, the total volume of the solution being about 250 ml. The precipitate was treated as above and the filtrate tested for bismuth.

The results presented in Table II indicate the importance and necessity of an extremely careful neutralization of the bismuth nitrate solution prior to the addition of sodium formate. The hydrogen-ion concentration should not be below pH 2, and the addition of extra formic acid, suggested by Benkert and Smith, should be omitted. The data in Table II prove that, provided the acidity of the solution is carefully adjusted, fairly good but slightly low results are obtained with Benkert and Smith's method as far as the precipitation of bismuth is concerned.

Table II. Effect of Initial Acidity

(200 mg. of bismuth used)

8 *N* HNO_3 Added	Approximate H^+ Concentration	Bi Found in Filtrate
Ml.		*Mg.*
0.1	2.5	0.2
0.3	2.0	0.2
0.5	1.8	0.5
0.6	1.7	0.6
0.7	1.6	0.8
0.9	1.5	1.0
1.2	1.4	1.2
1.5	1.3	1.3

Table III. Separation of Bismuth from Lead

Bismuth Found						Lead Found		
Bi Used	In ppt.	Corrected value	In 2 Filtrates of $PbSO_4$ Ppt. I	In 2 Filtrates of $PbSO_4$ Ppt. II	Pb Used	In 2 Filtrates of Bi Formate I	In 2 Filtrates of Bi Formate II	In Bi_2O_3 ppt.
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
10	9.9	9.8	0.10	0.12	25	22.7	2.2	0.1
20	19.8	19.5	0.19	0.22	50	46.3	3.4	0.3
20	19.9	19.4	0.32	0.26	100	92.3	7.0	0.5
50	50.1	49.1	0.31	0.34	100	90.4	8.7	1.0
50	51.6	49.1	0.31	0.29	200	185.9	11.6	2.4
100	102.0	99.0	0.34	0.34	200	184.0	12.2	3.0
100	104.0	99.1	0.32	0.33	500	462.7	31.8	4.9
300	302.8	298.8	0.46	0.41	200	182.3	13.5	4.0
300	305.2	299.0	0.43	0.40	500	450.0	43.2	6.2
					Grams			
300	309.0	299.1	0.32	0.35	1	907.6	80.9	10.0
25	25.1	24.9	Not determined		20	Not determined		0.2
50	53.7	49.7	Not determined		20	Not determined		4.0
100	107.0	100.2	Not determined		20	Not determined		6.8
200	209.4	199.8	Not determined		20	Not determined		9.6
300	312.8	300.4	Not determined		20	Not determined		12.4
300	311.2	300.1	Not determined		5	Not determined		11.1

To test the separation of bismuth from lead, the writer dissolved varying amounts of the two metals in nitric acid and determined bismuth with the above method, using all precautions during the neutralization process and employing two formate separations. Lead was determined separately in the two filtrates of the bismuth formate, by precipitating and weighing it as the sulfate. The bismuth oxide was tested for lead and the filtrates of the lead sulfate were tested for bismuth.

The results in Table III show conclusively that Benkert and Smith's method does not yield a clear-cut separation of bismuth from lead, particularly when large amounts of the latter are involved and only two sodium formate separations are employed. This is, in the writer's opinion, mostly due to the failure to expel by boiling all carbon dioxide (resulting from neutralizing nitric acid with sodium carbonate) prior to the addition of sodium formate. With increasing amounts of lead the influence of formic acid upon the complete precipitation of bismuth diminishes and is negligible when 5 grams of lead are present. This will facilitate considerably the determination of small amounts of bismuth in test or refined lead, or in lead buttons obtained by scorifying or fusing ores.

The major factors which affect the efficiency and accuracy of Benkert and Smith's method pointed out in detail above, make it appear that the method in its original form is incompatible with accuracy. Hence the present writer has worked out a modified scheme of analysis, incorporating the best features of the above method but obviating the attending difficulties.

New Procedure

This method is suited both for the quantitative separation and determination of bismuth and lead, and for the quantitative separation of small amounts of bismuth from large amounts of lead (10 to 50 grams). No other method has this particular advantage.

Extreme care is given to the neutralization of the bismuth and lead nitrate solution when bismuth has to be separated from small or moderate amounts of lead and the hydrogen-ion concentration of the solution should not be lower than that equivalent to pH 1.7. An extremely careful neutraliza-

tion is less important when more than 5 grams of lead are present.

Extensive tests revealed that basic bismuth formate is slightly soluble in sodium salts in general, and in sodium formate in particular (0.1 to 0.4 mg. of bismuth was colorimetrically found in the filtrate of varying amounts of bismuth formate which had been treated with 6 to 10 grams of sodium formate). Therefore, all sodium salts are replaced by ammonium salts. The use of ammonium salts has another important advantage in preventing the contamination of the bismuth formate precipitate by sodium salts. This in turn enables the elimination of the objectionable carbonate precipitation of bismuth with ammonium carbonate. Bismuth formate can be ignited directly to the oxide in the same way as the carbonate. This procedure is new and reduces considerably the time required to perform the separation and determination of bismuth and lead.

If a simultaneous lead determination is not required, the precipitation of the bismuth as basic formate can be connected with a final determination as the oxychloride, only one formate separation being necessary.

If only minute amounts of bismuth are present, the basic formate precipitate can be dissolved in hot dilute sulfuric acid and bismuth determined colorimetrically with potassium iodide.

The final determination of lead with this method is equally simple and easy of manipulation. The two filtrates from the bismuth formate precipitate are combined, nitric acid is added, and the formic acid is expelled by boiling and evaporation. Ammonium acetate is added and lead is finally precipitated as lead chromate with ammonium or potassium dichromate.

Experimental Procedure. Reagents required: nitric acid, 8 *N;* ammonia, 4 *N* and 6 *N;* ammonium carbonate solution, 5 per cent; and ammonium formate solution, about 40 per cent. Dilute 300 ml. of 90 per cent formic acid with 100 ml. of water. Neutralize with 12 *N* ammonia, using litmus paper as indicator (about 650 ml. of ammonia are required).

Neutralize the warm nitric acid solution of bismuth and lead (containing not more than 400 mg. of bismuth) with 4 *N* ammonia until a permanent precipitate develops. Add 8 *N* nitric acid, drop by drop, until the precipitate just dissolves. Neutralize excess nitric acid carefully with 5 per cent ammonium carbonate until further addition would cause precipitation of basic bismuth carbonate. The solution should be definitely clear at this stage.

Heat the solution to boiling and hold at that point for 5 minutes to expel all carbon dioxide. Disregard any precipitate which forms during the heating. Add 7 to 8 ml. of ammonium formate solution, heat to boiling, and allow to stand on a hot plate or water bath for about 15 minutes. (If as little as 1 mg. or less of bismuth is present, allow to stand for about 2 hours.)

Filter through No. 42 Whatman or similar paper and wash precipitate 5 times with hot water. Wash precipitate back into original beaker and dissolve in 5 to 6 ml. of 8 *N* nitric acid.

Repeat the ammonium formate separation and filter through original paper into original filtrate, washing the precipitate 8 times with hot water. Retain filtrates. Wash precipitate once with alcohol. Ignite cautiously in a weighed porcelain crucible. The carbon of the paper is likely to have a reducing action on the precipitate; therefore, after the paper is burned off, cool the crucible and moisten the precipitate with 8 *N* nitric acid, dry on a hot plate, and ignite cautiously to the oxide, Bi_2O_3.

Add 30 ml. of nitric acid to the combined filtrates of the bismuth formate, evaporate to a small volume (20 to 30 ml.) until heavy white fumes escape, and add 5 ml. of 16 *N* nitric acid and 30 ml. of 33 per cent ammonium acetate solution. Dilute to 250 ml., heat to boiling, add an excess of hot ammonium or potassium dichromate solution and boil for 2 to 3 minutes until the precipitate turns to a shade of orange. Filter on a weighed Gooch crucible, wash with hot water, dry at 105° C., and weigh as lead chromate.

Bismuth in Lead. To determine bismuth in lead bullion and refined lead, and in lead buttons, obtained by scorifying or fusing ores, place 10 to 50 grams of lead in a 600-ml. beaker, dissolve in nitric acid (1 to 4), and neutralize the warm solution with 6 *N* ammonia until further addition would cause precipitation of lead and bismuth. The solution should be definitely clear at this point, but the acidity should not exceed 0.05 *N*—that is, 2 ml. of 8 *N* nitric acid in 300 ml. of solution. Add 7 to 8 ml. of ammonium formate solution, allow to stand, and settle on hot plate. As little as 1 mg. of bismuth will separate in one hour. Filter off precipitate and wash 8 times with hot water. Dissolve with warm 4 *N* hydrochloric acid into original beaker, almost neutralize with 6 *N* ammonia, and dilute with hot water to 500 ml. Allow to settle, filter on a weighed Gooch crucible and wash with hot water, dry at 105° C., and weigh as bismuth oxychloride.

Colorimetric Determination of Bismuth (less than 0.7 mg.). Dissolve the bismuth formate precipitate in hot sulfuric acid (1 to 3), transfer the solution (or an aliquot portion) to a 50-ml. Nessler tube, add 2 or 3 drops of dilute sulfur dioxide water, then about 2 grams of potassium iodide, and make up to the 50-ml. mark. Compare in a colorimeter with another tube containing the same amounts of sulfurous acid and potassium iodide and the same volume of solution. Add to this tube a standard bismuth sulfate solution (containing 0.1 gram of bismuth per liter) until the colors of the two solutions are the same depth.

Corroboration and Verification

Varying quantities of bismuth and lead have been determined with the method described above, by adjusting the acidity of the nitric acid solution of the two metals and proceeding as shown in "Experimental Procedure". The accuracy of the method is indicated by Table IV.

Table IV. Accuracy of Proposed Method

Bi Used	Bismuth Found		Lead Used	Lead Found	
	Original	Duplicate		Original	Duplicate
Mg.	*Mg.*	*Mg.*	*Grams*	*Mg.*	*Mg.*
0.5[a]	0.48	0.50	20	Not determined	
1.0[a]	0.99	0.96	20	Not determined	
10.0[b]	9.9	9.8	100 mg.	99.7	99.7
20.0[b]	19.6	20.0	100 mg.	99.8	99.6
50.0[b]	49.7	49.7	100 mg.	99.6	99.7
50.0[c]	49.9	49.8	20	Not determined	
100.0[b]	99.8	99.6	300 mg.	299.3	299.5
100.0[c]	100.0	99.7	20	Not determined	
200.0[b]	199.5	199.9	500 mg.	499.2	500.1
300.0[b]	299.9	300.2	1000 mg.	1000.3	1000.2
300.0[c]	300.3	299.6	20	Not determined	

[a] Bismuth determined colorimetrically, [b] as oxide, [c] as oxychloride.

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Determination of Small Quantities of Fluoride in Water

A Modified Zirconium-Alizarin Method

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METHODS for the determination of fluoride reviewed in a report (*1*) prepared in 1941 by the American Water Works Association Committee on Methods of Determining Fluorides were not found satisfactory for the routine analysis of a large number of samples of natural waters that generally contain less than 0.4 part per million of fluoride. A report (*4*) published in 1941 by the United States Public Health Service, pointing out that small quantities of fluoride in water may inhibit dental caries, has given added significance to the accurate determination of small quantities of fluoride.

Using the zirconium-alizarin indicator of de Boer (*2*), methods of determining fluoride, or modifications, have been developed by Casares and Casares (*3*), Thompson and Taylor (*8*), Elvove (*5*), Sanchis (*6*), and Scott (*7*). In these methods the waters were acidified with hydrochloric acid or with hydrochloric and sulfuric acids. It is known that when the color of the zirconium-alizarin indicator is developed with hydrochloric acid, the effect of sulfate present in the water must be taken into account. When the waters are acidified with both hydrochloric and sulfuric acids the interference of sulfate is less than when hydrochloric acid alone is used, but tests have confirmed the statement of Walker and Finlay (*9*) that sulfate over about 120 parts per million interfered with the colorimetric determination of fluoride by the zirconium-alizarin method when both hydrochloric and sulfuric acids were used. By the procedure reported here the waters are acidified with sulfuric acid, thereby decreasing the interference of sulfate to the extent that waters containing less than 500 parts per million of sulfate may be analyzed for fluoride without resorting to a correction curve. The alkalinity of a sample may interfere with the fluoride determination by its effect on the pH of the solution. This effect is negligible up to about 1000 parts per million of bicarbonate in methods (*5*) using fairly strong hydrochloric acid. The bicarbonate should be neutralized if it is more than about 150 parts per million for methods using hydrochloric and sulfuric acids at a lower concentration (*6*). Neutralization of the alkalinity with nitric acid as prescribed in the proposed method eliminates any uncertainty due to the alkalinity of the sample. The zirconium-alizarin indicator appears to be more stable in sulfuric acid than in hydrochloric acid or in a mixture of hydrochloric and sulfuric acids.

Low or moderate quantities of fluoride in water can be measured conveniently and accurately by selecting the appropriate concentrations of the reagents.

Reagents

PREPARATION OF INDICATOR. (1) Zirconyl nitrate, 1.75 grams of $ZrO(NO_3)_2.2H_2O$ in 250 ml. (2) Alizarin red, 0.35 gram of alizarin monosodium sulfonate in 250 ml. (3) When the indicator is needed add 10 ml. of the zirconyl nitrate solution to approximately 50 ml. of distilled water, then add slowly with constant stirring 10 ml. of the alizarin solution and make up to a volume of 200 ml. with distilled water. The indicator should be stored in the dark and it should be used before it is more than a few days old.

Sulfuric acid, 2.10 N ($\pm$0.02 N).

Nitric acid, 0.1639 N (1 ml. will neutralize 10 mg. of HCO_3).

Sodium fluoride. Stock solution, 0.2210 gram of sodium fluoride in 1 liter. Standard solution, dilute 100 ml. of the stock solution to 1 liter (1 ml. equals 0.01 mg. of fluoride).

Procedure

Transfer 100 ml. of the clear samples to be analyzed to comparison tubes, neutralize the alkalinity with the 0.1639 N nitric acid (neutralization not necessary for waters containing less than 100 parts per million of bicarbonate), make up to a volume of 105 ml., and add exactly 5 ml. of the 2.1 N sulfuric acid. Transfer to a series of comparison tubes 0, 2, 4, 6, 8, 10, and 12 ml. of the standard fluoride solution (1 ml. equals 0.01 mg. of fluoride), make up to a volume of 105 ml., and add exactly 5 ml. of the 2.1 N sulfuric acid. Add 5 ml. of the zirconium-alizarin indicator to each sample and standard, mix well, and allow to stand overnight. An inspection made promptly after the indicator has been added will show any samples out of the range of the standards. Take smaller quantities of water for any samples that appear to contain more fluoride than the highest standard. After the samples and standards have stood about 18 hours, mix well, and compare in a 3-hole colorimeter in which each sample is compared with the two nearest standards.

Discussion of Method

When the samples of water to be analyzed are acidified with 5 ml. of 2.1 N sulfuric acid a good color range is obtained for amounts of fluoride ranging from 0.0 to 0.12 mg. For waters containing more than about 1.2 parts per million of fluoride, samples smaller than 100 ml. should be used. For waters containing appreciably higher fluoride the samples and standards may be acidified with exactly 5 ml. of 1.8 N sulfuric acid. When 1.8 N sulfuric acid is used a good color range is obtained for amounts of fluoride ranging from 0.04 to 0.18 mg. In either case the fluoride may be read to 0.01 mg. within the ranges indicated.

Although satisfactory results are sometimes obtained when comparisons are made at considerably less or more than 18 hours, comparisons at about 18 hours are likely to give the best results. For samples and standards that have stood about 18 hours it is found that as much as an hour difference in time of adding the reagents does not appreciably affect the determination. This fact can be used to advantage when any sample is shown by prompt inspection to be out of the range of the standards. A smaller sample can be taken, so that when comparisons are made after about 18 hours all the samples will be within the range of the standards.

Studies of the major ions usually found in natural waters have shown that sulfate causes the most interference. By acidifying the samples with sulfuric acid the interference of sulfate is reduced. On the basis of 100-ml. samples the error that may be introduced by sulfate or chloride is as follows: 500 parts per million of sulfate are equivalent to about +0.01 mg. of fluoride and 1000 parts per million of chloride are equivalent to about −0.01 mg. of fluoride. Since the errors introduced by sulfate and chloride are plus and minus, respectively, the effect of the one will tend to counteract the effect of the other. The effect of nitric acid added to neu-

tralize the alkalinity and of nitrate usually found in natural waters is negligible.

Summary

The zirconium-alizarin method has been modified to facilitate the convenient and accurate determination of small amounts of fluoride in a large number of water samples. Sulfuric acid is used to acidify the samples to reduce the interference of sulfate. The pH is accurately controlled to give the most sensitive comparisons. Most natural waters can be analyzed by the modified procedure without resorting to correction curves. The fluoride content of waters containing less than 500 parts per million of sulfate, 500 parts per million of bicarbonate, and 1000 parts per million of chloride may be determined within a limit of about 0.1 part per million when a 100-ml. sample is used.

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Determination of Melibiase Activity

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IN SUGAR analysis, methods which are based on enzyme hydrolysis are recognized (*6*, *7*) to possess an inherent accuracy superior to customary acid hydrolysis methods. A reason why such invertase methods have not been more generally adopted may be an impression (*6*, *7*) that each laboratory must first laboriously prepare and standardize suitable invertase preparations from yeast. Highly active invertase preparations specifically suited for analytical purposes, however, have been available for a number of years.

In the case of beet sugar refinery control and generally in the determination of sucrose and raffinose, a further difficulty is the lack of a satisfactory procedure for determining the melibiase activity of the enzyme preparations as a basis for their standardization.

Invertase hydrolyzes raffinose to produce levulose and melibiose. The melibiose thus formed may then be hydrolyzed by the enzyme melibiase into its constituent monosaccharides, galactose and glucose. This has been the basis of methods for determining raffinose and sucrose for many years (*3*, *5*, *8*). Bau (*2*) as well as Fischer (*4*) established long ago that bottom-fermenting yeast contains melibiase, whereas top-fermenting yeast does not. Based upon this fact, methods were devised for the determination of raffinose by differential fermentation with the two yeasts.

The literature contains few references to the determination of the activity of the enzyme melibiase.

Reynolds (*9*) was concerned mainly with the preparation of the enzyme; nevertheless, he refers to the unimolecular reaction velocity constant as a measure of the activity of his melibiase preparations. The course of hydrolysis was followed by observing the changes in polarization of a 20° V. melibiose solution. Since the total range of polarization between 0 and 100 per cent hydrolysis is only from +18.2° to +9.1° V., the method is not very sensitive. Pure melibiose being unobtainable at the time, it was necessary to prepare the melibiose from raffinose by fermentation with top-fermenting yeast.

The present A. O. A. C. (*1*) procedure for raffinose determination employs enzyme preparations as prepared from the two types of yeast, invertase with and without melibiase. This is based on the work of Hudson and Harding (*5*) and Reynolds (*9*), and particularly the comprehensive paper by Paine and Balch (*8*).

The method requires that the enzyme solution employed for the hydrolysis of raffinose solutions be capable of hydrolyzing 10 times its volume of 20° V. melibiose solution in 30 minutes at 20° C. to specified degrees of hydrolysis, depending on the amount of raffinose present in the material to be analyzed. The polarizations in degrees Ventzke corresponding to the percentages of hydrolysis which are shown, however, do not allow for dilution of the melibiose by the enzyme. Actually, after such correction, the polarizations equivalent to 35, 50, and 70 per cent hydrolysis would be 15.0, 13.6, and 11.6° V., respectively, instead of 16.4, 14.9, and 12.9° V. as indicated. A considerable difference, amounting to almost 100 per cent of the concentration of enzyme to be employed, is involved in the differences between the two series of polarization values.

In any case, the A. O. A. C. method represents merely a means of ascertaining approximately whether a minimum of enzyme activity will be present to ensure the complete hydrolysis of the raffinose present at each level and is not represented to be a method for expressing by a numerical value the melibiase activity of an enzyme preparation.

Weidenhagen (*11*, *12*) described a method in which the melibiose hydrolysis is followed by determining the changes in reducing action towards Fehling's solution and subsequent titration of the reduced copper with permanganate. This method also has the disadvantage of having only a limited range and hence limited sensitivity (18.7 to 10.5 ml. of potassium permanganate).

In 1932, Tauber and Kleiner (*10*) published a method for determining monosaccharides in the presence of reducing disaccharides, pointing to its probable usefulness in studying saccharases and glycosidases. Their method involves selective reduction of a Barfoed solution, modified by addition of lactic acid,

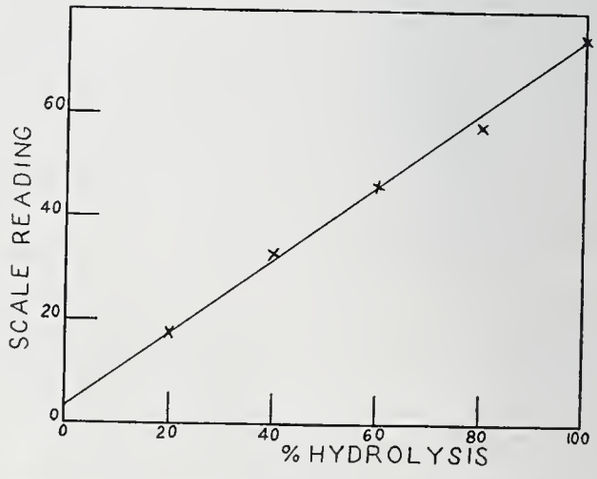

Figure 1. Relationship between Per Cent Hydrolysis and Scale Reading

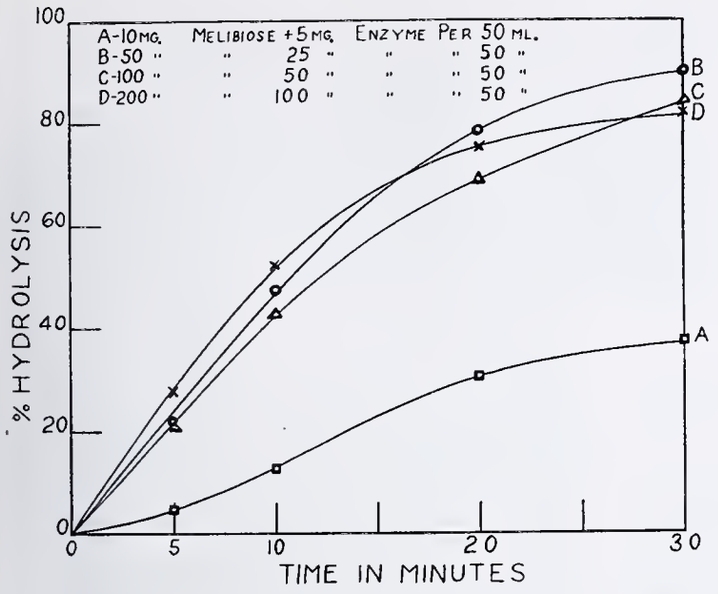

FIGURE 2. EFFECT OF CONCENTRATION ON HYDROLYSIS

and then permitting the cuprous copper formed to reduce an acid molybdate solution, the resulting blue color being measured photometrically. Because of the greatly increased sensitivity possible with relatively minute amounts of material, as compared with existing rotation methods, the procedure appeared excellently suited for following the hydrolysis of melibiose by melibiase. At about this time pure melibiose also became available.

These facts made it possible to investigate the course of the hydrolysis of melibiose as a basis for a method for determining the activity of melibiase preparations used in the analysis of raffinose-containing materials.

Calibration Hydrolysis Curve

Mixtures of melibiose, glucose, and galactose representing the products present in the course of the hydrolysis were prepared to correspond to varying hydrolysis percentages. After the reduction, photometer readings (an American Instrument Co. neutral wedge photometer was used) in a 1.25-cm. (0.5-inch) cell were taken, using a 6100 Å. filter (since maximum absorption is obtained in the red portion of the spectrum). Because of the tendency of the color to fade, readings were made 2 minutes after dilution.

Figure 1 shows the linear relationship between scale readings and per cent hydrolysis, permitting calculation of per cent hydrolysis corresponding to any photometer reading as follows:

$$\% \text{ hydrolysis} = \frac{S_x - S_0}{S_{100} - S_0} \times 100$$

where S_x = scale reading obtained at a given stage in hydrolysis
S_0 = scale reading at 0 per cent hydrolysis, and
S_{100} = scale reading at 100 per cent hydrolysis

Conditions of Hydrolysis

The following test conditions were employed for measuring the hydrolysis:

The substrate, except where otherwise indicated, consisted of 1.104 grams of melibiose hydrate (equivalent to 1.000 gram of anhydrous melibiose) and 5 ml. of Walpole's acetate pH 4.5 buffer diluted to 100 ml. Phosphate buffers may not be used because of subsequent precipitation with the copper reagent.

Ten milliliters of substrate and 25 ml. of water were attemperated at 20° C. and then 5 ml. of the enzyme solution were added. At the end of the specified time of hydrolysis, 2 ml. of a sodium carbonate solution, containing 2 grams per 100 ml., were added to stop the hydrolysis and the volume was adjusted to 50 ml. with water. Ten milliliters of the hydrolysis mixture were diluted to 100 ml., and the monosaccharides were determined on a 5 ml. aliquot as in the method described below.

The enzyme preparation used in the preliminary work was an invertase preparation in scale form similar to the product regularly available for analytical use and obtained by precipitation and purification from bottom yeast autolyzates.

Hydrolysis at Varying Concentrations

A few preliminary tests were conducted to determine the most satisfactory concentration of melibiose to employ to permit a study of the hydrolysis reaction. Melibiose concentrations were varied from 10 mg. per 50 ml. to 200 mg. per 50 ml. (final volume), the ratio of enzyme to substrate remaining constant at 1 to 2. Figure 2 represents the course of hydrolysis for these hydrolysis mixtures.

A concentration of 100 mg. per 50 ml. represented by curve C appears to represent a satisfactory melibiose concentration for such studies and further tests were carried out accordingly on this basis.

TABLE I. HYDROLYSIS AT VARYING ENZYME CONCENTRATIONS

Enzyme Concentration G./100 ml.	Time Min.	Hydrolysis %	k^a
0.1	15	14.2	0.0044
	30	21.3	0.0035
	45	34.3	0.0041
	60	40.0	0.0037
	90	57.3	0.0041
			Av. 0.0040
0.2	15	21.0	0.0068
	30	42.5	0.0080
	45	57.1	0.0082
	60	69.3	0.0085
	90	82.5	0.0084
			Av. 0.0080
0.4	15	42.2	0.0159
	30	68.6	0.0164
	45	82.5	0.0168
	60	94.6	0.0211
	90	97.5	0.0178
			Av. 0.0176

[a] $k = \frac{1}{t} \log_{10} \frac{a}{a - x}$.

Hydrolysis with Varying Amounts of Enzyme

Reynolds and Weidenhagen have referred to the unimolecular reaction velocity constant, k, as a measure of melibiase activity. Table I contains hydrolysis data including the velocity constants, k, obtained. This is represented also by Figure 3, showing the course of hydrolysis at three

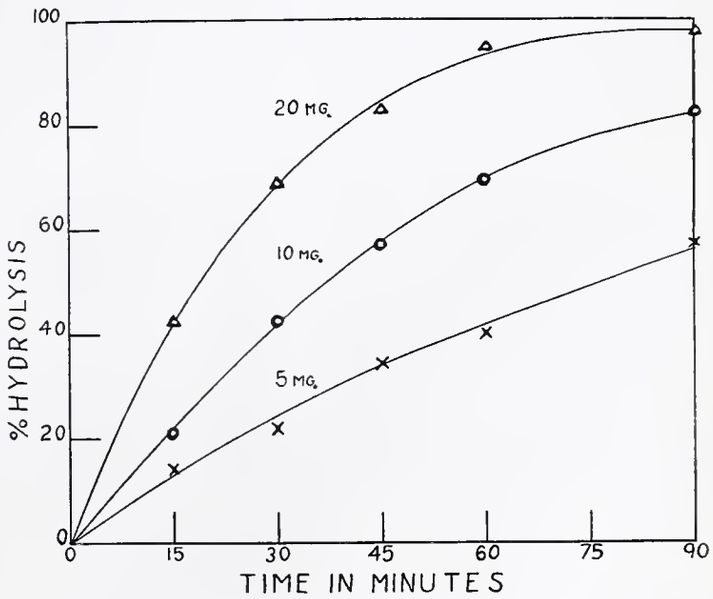

FIGURE 3. HYDROLYSIS AT VARYING ENZYME CONCENTRATIONS

Table II. Proportionality of Enzyme Concentration with k Values as Obtained from 30-Minute Hydrolysis

Enzyme Concentration G./100 ml.	Hydrolysis %	Activity of Diluted Enzyme k	Activity of Original Enzyme k value[a]
0.10	14.6	0.0023	2.3
0.20	30.6	0.0053	2.6
0.30	44.1	0.0084	2.8
0.40	55.9	0.0119	3.0
0.50	63.9	0.0147	2.9
0.70	79.0	0.0229	3.3
1.00	89.2	0.0322	3.2

[a] Represents velocity constant, k, multiplied by dilution factor—for enzyme concentration of 1 gram per 100 ml., k value = $k \times 100$.

Table III. Hydrolysis of Sucrose and Raffinose with Varying Amounts of Melibiase

Melibiase Activity k	Amounts Taken: Sucrose G./100 ml.	Amounts Taken: Raffinose (anhydrous) G./100 ml.	Direct Polarization ° V.	Invert Polarizations: Invertase ° V.	Invert Polarizations: Invertase + melibiase ° V.	Amounts Found: Sucrose G./100 ml.	Amounts Found: Raffinose G./100 ml.
0.01	13.00	0.20	+51.38	−15.20	−15.62	13 00	0.15
0.05					−15.65	12.98	0 16
0.10					−15.75	12.96	0.19
0.20[a]					−15.68	12.98	0.17
0.02	13.00	0.65	+54.34	−13.60	−14.23	13.20[b]	0.22[b]
0.10					−15.27	12.98	0.59
0.20					−15.16	12.99	0.55
0.35[a]					−15.26	12.98	0.59
0.04	13.00	1.30	+59.20	−11.30	−12.75	13.52[b]	0.51[b]
0.20					−14 27	13.17[b]	1.05[b]
0.40					−14.77	13 05	1.23
0.60[a]					−14.75	13.05	1.22

[a] Enzyme activities recommended by A. O. A. C.
[b] Incomplete hydrolysis.
Invertase activity more than 0.1 (k value) in all tests.

enzyme concentrations under the conditions described. As in the case of the application of such k values to invertase the reaction appears to follow a unimolecular course within limits.

For simplicity, it would be desirable for the determination of melibiase activity of unknown preparations to involve determination of per cent hydrolysis after a single hydrolysis period. Accordingly, Table II shows calculated k values obtained when varying amounts, from 5 to 50 mg., of a particular enzyme preparation were employed in the hydrolysis of a melibiose solution for exactly 30 minutes and the per cent hydrolysis and corresponding k values obtained. A satisfactory linear relationship between enzyme concentration and k value is shown to exist between the limits of 30 and 70 per cent hydrolysis.

Based on these data, the use of calculated k values for expressing melibiase activity appears justified under the prescribed test conditions.

The method in detail is described below. The monose determination, which is essentially the method of Tauber and Kleiner, is included for convenience.

Enzymatic Hydrolysis

Melibiose Substrate. Dissolve 1.104 grams of melibiose hydrate (obtained from the Eastman Kodak Co., Rochester, N. Y.) in water, add 5 ml. of Walpole acetate pH 4.5 buffer, and dilute to 100 ml. One ml. = 10 mg. of anhydrous melibiose. (The buffer solution is prepared by diluting 114 ml. of 1 M acetic acid and 86 ml. of 1 M sodium acetate to 1000 ml.)

Hydrolysis. To 10 ml. of substrate solution in a 50-ml. volumetric flask, add 25 ml. of water and hold in a 20° C. water bath for at least 10 minutes. Add 5 ml. of enzyme solution of appropriate concentration (adjusted to 20° C.) and start stop watch at introduction of enzyme. After exactly 30 minutes, add 2 ml. of sodium carbonate solution (2 grams of sodium carbonate per 100 ml.) to stop the reaction. Make up to volume, dilute 10 ml. to 100 ml., and determine monosaccharides on a 5-ml. aliquot in duplicate.

Determination of Degree of Hydrolysis

Reagents. *Acid Copper Reagent.* Dissolve 48 grams of copper acetate (Merck, normal, c. p.) in 850 ml. of boiling water contained in a beaker. (Remove from flame before adding copper salt.) Add 50 ml. of 8.5 per cent lactic acid to the hot solution. Any precipitate that may have formed should dissolve upon stirring. Cool, dilute to 1000 ml., and filter.

Molybdenum Color Reagent. Place 150 grams of molybdic acid anhydride (free from ammonia) and 75 grams of pure anhydrous sodium carbonate in a 2-liter Erlenmeyer flask and add water in small portions until 500 ml. have been added. (Caution: The water should be added slowly and the flask shaken continuously, as considerable carbon dioxide and heat are evolved.) Heat to boiling or until all of the molybdic acid has been dissolved. Add 300 ml. of 85 per cent phosphoric acid, cool, dilute to 1000 ml., and filter.

Determination. Pipet 5 ml. of the sugar solution into a test tube (preferably about 15 × 200 mm.) and add 5 ml. of the copper reagent. Place tube in a boiling water bath (containing double bottom) and start stop watch. After exactly 8 minutes remove and place in a 20° C. water bath. Hold 5 minutes at 20° C. (13 minutes on watch), and add 5 ml. of the molybdenum color reagent. Mix by lateral shaking and after 2 minutes transfer to a 50-ml. volumetric flask, rinse, and dilute to mark. (If color is too intense, standardize the test for dilution to a higher volume.) Read in photometer, using a red filter, within 2 to 3 minutes after dilution (17 to 18 minutes on stop watch), as the color fades fairly rapidly. Since the method is empirical, conditions must be closely adhered to.

Calculation of k Value. Determine the photometer reading for the 0 and 100 per cent hydrolysis. The 0 per cent hydrolysis is represented by the solution of the enzyme added to the substrate after addition of the sodium carbonate. The 100 per cent hydrolysis is obtained by hydrolyzing the substrate with sufficient enzyme overnight at room temperature.

Calculate the per cent hydrolysis as indicated above and the equivalent unimolecular constant:

$$k = \frac{1}{t} \log_{10} \frac{100}{100 - x}$$

where x = % hydrolysis

Example. 0.2% enzyme solution used in hydrolysis

$S_x = 37.9$
$S_0 = 10.6$
$S_{100} = 74.9$

$$\% \text{ hydrolysis} = \frac{37.9 - 10.6}{74.9 - 10.6} \times 100 = 42.5$$

$$k = \frac{1}{30} \log_{10} \frac{100}{100 - 42.5} = 0.0080 \text{ (activity of enzyme solution as used for hydrolysis)}$$

$$k \text{ value of original enzyme} = \frac{100}{0.2} \times 0.008 = 4.0$$

Table IV. Melibiase Activities of Brewers' Yeasts

Number	Type of Yeast	k Value (Dry Yeast)
1	Ale	Nil
2	Lager	0.70
3	Lager	0.24
4	Ale	Nil
5	Lager	0.85
6	Lager	0.76
7	Lager	0.25
8	Lager	0.95

Application to Raffinose Determinations

The difficulty encountered in the proper interpretation of the A. O. A. C. procedure for determining the amount of enzyme to use at varying expected raffinose levels has been referred to.

It would, no doubt, be of advantage to establish the activity requirements of melibiase in terms of k values. Experiments which were made to establish the relationship between k values by the proposed method and per cent hydrolysis as determined by the A. O. A. C. procedure indicate that enzyme solutions of k values of 0.2, 0.35, and 0.6 are required to yield respectively, 35, 50, and 70 per cent hydrolysis under the A. O. A. C. conditions.

It was also considered of interest to determine the melibiase activities actually required to bring about overnight complete hydrolysis of various amounts of raffinose at 20° C. Three sugar mixtures, each containing 13 grams of sucrose and 0.2, 0.65, and 1.3 grams of raffinose per 100 ml., respectively, were hydrolyzed according to the method of the A. O. A. C. with various amounts of enzyme solution of known activities. The results, as shown in Table III, indicate that the present A. O. A. C. requirements involve a considerably larger excess of melibiase than is actually necessary. Further investigation might profitably be conducted in this connection.

Activities of Various Yeasts

Using this procedure, the melibiase activities (k value) were determined on a series of yeasts as obtained from a number of breweries and representing both ale (top) and lager (bottom) yeasts in use (Table IV). In determining the enzyme activity of live yeast, consideration must be given to the procedure used for extracting the enzyme. The preparation of invertase from yeast usually involves some means of autolysis of the yeast, toluene being the most common reagent referred to. Weidenhagen, in investigating melibiase activities as obtained with different autolyzing solvents, found that ethyl acetate appeared to give the most satisfactory result. This has been confirmed, and accordingly ethyl acetate was used in these tests.

Determination of Melibiase Activity of Yeast

Drain the liquid yeast as received, on a Büchner funnel, and wash several times with cold water. Plasmolyze 5 grams of the yeast cake by adding 1 ml. of ethyl acetate and stir intermittently for 10 minutes. Add 5 ml. of water, stir well, and after 5 minutes dilute to 100 ml. Use 5 ml. of this suspension directly for the hydrolysis as described above. Filter immediately after addition of sodium carbonate at end of hydrolysis and proceed as directed. Make a blank determination on another portion of substrate by adding sodium carbonate prior to the yeast and then continuing as above.

The procedure represents also a convenient rapid chemical method for distinguishing between ale and lager yeasts.

Summary

A new method for the determination of the activity of the enzyme melibiase utilizes the Tauber and Kleiner procedure for determining monosaccharides in the presence of disaccharides as a means of following the course of hydrolysis of melibiose. The hydrolysis has been found to follow a unimolecular course within limits and hence the activities have been expressed as k values as determined after a 30-minute hydrolysis period. Experiments made on various sucrose-raffinose mixtures indicate that the amounts of enzymes specified by the A. O. A. C. for hydrolysis are probably unnecessarily high. A method for determining the melibiase activity of yeast is described and the values for a number of brewery yeasts are given; the method has also been found suitable for distinguishing between top- and bottom-fermenting yeasts.

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Method for Determination of Ethyl Alcohol for Medicolegal Purposes

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CONSIDERABLE interest exists regarding methods for the determination of ethyl alcohol in body fluids, since the alcohol content of these fluids is used to determine the degree of inebriety. Most of the methods proposed depend upon the direct oxidation of the volatile substances separated from the body fluids by aeration or distillation from an acid medium. Direct aeration or distillation from an acid medium must necessarily assume the presence of only relatively small amounts of other volatile substances which might reduce the oxidizing agent and be interpreted as alcohol.

Heise (7) reports that other reducing substances are not present in sufficient quantity to increase the alcohol reading significantly. However, sufficient formaldehyde or acetone may be present to increase the alcohol reading to a level considered intoxicating. Methenamine is commonly used as an urinary antiseptic and significant quantities of formaldehyde appear in the urine after its administration. While acetone is not oxidized quantitatively under the conditions commonly employed, its presence does increase the readings for alcohol.

In medicolegal work, the state of health of the individual or whether or not some kind of medication had been taken is not always known. Consequently, there always exists the possibility of the presence of other volatile substances, such as aldehydes, acids, phenols, and acetone. Since ethyl alcohol is the only alcohol ordinarily present to any appreciable extent in beverages, it is not so imperative that the method distinguish between the different alcohols as it is to separate the ethyl alcohol quantitatively from the other interfering substances. Where it may be necessary to differentiate between the alcohols, the distillate obtained may be used for this particular purpose. It is more essential to simplify the procedure and to increase the specificity of the test without sacrificing accuracy than it is to have available a less specific microtechnique which requires only one or two drops of blood for the determination. The proposed microtechniques have the advantage that blood specimens can be obtained by skin pricks. However, for medicolegal purposes, it is desirable to have some of the specimen available for future checks, which would scarcely be feasible when the microtechnique is employed.

Gettler and Siegel (5) suggested isolation of the alcohol and determination of its physical constants; however, this technique

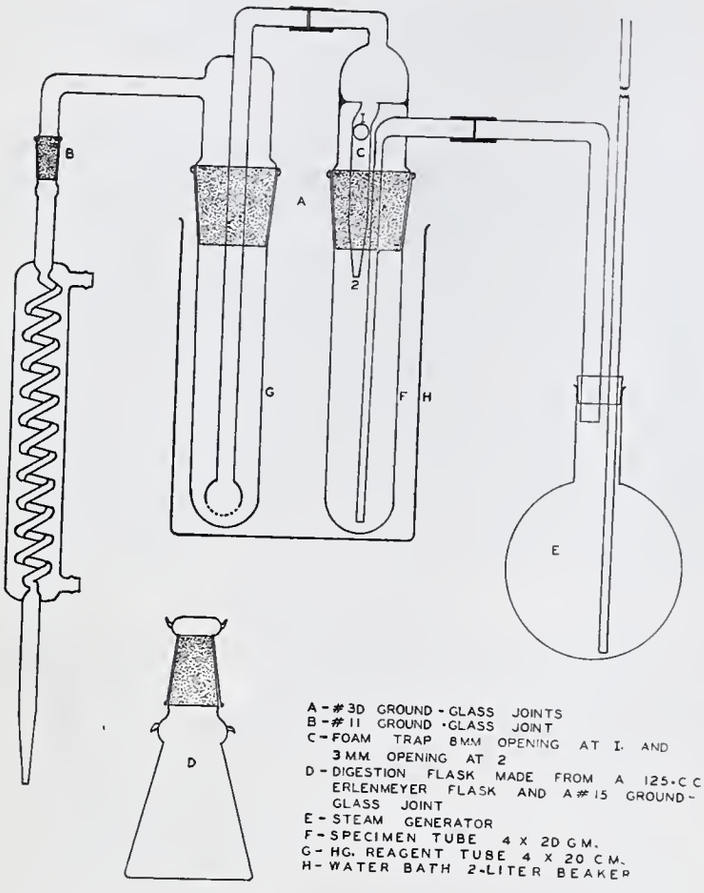
A - #30 GROUND-GLASS JOINTS
B - #11 GROUND-GLASS JOINT
C - FOAM TRAP 8MM OPENING AT 1 AND 3 MM OPENING AT 2
D - DIGESTION FLASK MADE FROM A 125-CC ERLENMEYER FLASK AND A #15 GROUND-GLASS JOINT
E - STEAM GENERATOR
F - SPECIMEN TUBE 4 X 200 MM.
G - HG. REAGENT TUBE 4 X 200 MM.
H - WATER BATH 2-LITER BEAKER

FIGURE 1. DISTILLATION APPARATUS

is intended for the determination of alcohol in tissues and relatively large quantities of material are required. Gorr and Wagner (*6*) studied the removal of aldehydes and ketones from alcohol solutions by refluxing the mixture with mercuric chloride in 1 *N* or 2 *N* sodium hydroxide for 2 to 5 hours and then distilling the alcohol. They found that the acetaldehyde and acetone were removed completely with recoveries of 94 to 96 per cent of the added alcohol.

A further study of the efficiency of mercuric oxide to remove aldehydes and ketones was made by Friedemann and Klaas (*4*), who substituted calcium hydroxide for sodium hydroxide. These authors suggest distilling the alcohol from an acid medium after precipitating the proteins with sodium tungstate and mercuric sulfate, and a second distillation from calcium hydroxide containing mercuric oxide to remove any volatile substances, other than alcohol, which were distilled from the acid medium. This technique requires a second transfer and distillation of the specimen, which is quantitatively undesirable for routine purposes.

The method described in this paper has been developed for routine analysis of specimens for medicolegal purposes. The authors found that the interfering substances are removed quantitatively by passing the distilled vapor from the acid medium through a solution of concentrated sodium hydroxide containing mercuric oxide. The alcohol is distilled and the interfering substances are removed in one operation, considerably simplifying the technique. It provides a simple means of separating the alcohol quantitatively from the other substances which may be present in biological material and dispenses with the necessity of testing for the presence of these contaminants. The acid medium removes any basic volatile substances, while the concentrated sodium hydroxide containing mercuric oxide removes the acids, aldehydes, acetone, and phenols. The method has the added advantage of distilling and digesting the specimen in an all-glass apparatus and hence prevents contamination from cork or rubber stoppers.

The authors have also observed that low recoveries are obtained by oxidizing the alcohol in an open container, undoubtedly owing to loss of between 10 and 20 per cent or even more acetaldehyde during the digestion. To prevent this loss, the alcohol is oxidized in a closed flask. The method of oxidizing the alcohol, or the determination of the excess oxidizing agent, is a matter of preference. Apparently all the reagents suggested for this phase of the determination give accurate results. However, the authors prefer to use potassium dichromate because the solution will keep indefinitely, which obviates the necessity of periodic restandardization. The excess dichromate is reduced with potassium iodide and the liberated iodine titrated with a 0.1 *N* sodium thiosulfate solution.

Apparatus

The distillation apparatus and the digestion flask are shown in Figure 1. The two tubes, *F* and *G*, are held in place during the distillation by springs attached to the top of the tubes and the side arms. The foam trap, *C*, as constructed will prevent the mechanical carrying over of material, even when considerable foaming occurs. The vapor passes through the opening at 1, and the condensate, together with any fluid forced through the opening, returns to the distillation tube through the opening at 2. The alcohol is collected from the condenser in flask *D*.

The male portion of the joint of tube *G* must be covered with graphite to prevent sticking. The graphite is best applied by using a carpenter's pencil, although an ordinary pencil will serve the purpose.

A 2-liter beaker may be used for the boiling water bath, *H*. The digestion flask, *D*, is constructed from a 125-cc. Erlenmeyer flask and a No. 15 interchangeable ground-glass joint. The cap is kept in place with two coil springs, 0.6 cm. (0.25 inch) in diameter, made from 20-gage spring steel wire. Considerable tension must be maintained on the springs to prevent the cap from being raised by the vapor pressure and breaking the cap or the flask when it snaps back into position. Two 10-cc. all-automatic burets are most convenient for the 0.1 *N* potassium dichromate and 0.1 *N* sodium thiosulfate solutions.

Reagents

Potassium dichromate solution, 0.1 *N*, 4.903 grams per liter, reagent quality. A solution prepared from reagent quality potassium dichromate agrees with the theoretical yield of alcohol by direct digestion of standard alcohol solutions. One cubic centimeter of the solution is equivalent to 1.15 mg. of alcohol. (A 0.1 *N* solution is preferred to more dilute solutions because it covers the range of the quantities of alcohol commonly found in the blood or urine specimens, and if reasonable care is exercised, excellent checks can be obtained even with as small quantities as 0.005 per cent.)

Sodium thiosulfate solution, 0.1 *N*, 25 grams of sodium thiosulfate plus 0.1 gram of sodium bicarbonate per liter. This solution is standardized against the 0.1 *N* potassium dichromate solution.

Potassium iodide crystals, analytical reagent, must be free from iodates.

Arrowroot starch, 1 per cent solution.

Sulfuric acid, concentrated, reagent quality.

TABLE I. RECOVERIES OF ALCOHOL FROM URINE AND BLOOD

Material	Sample	Alcohol Added	Acetone Added	Formaldehyde Present	Alcohol Recovered	
	Cc.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	%
Water	2.5	2.35	..	..	2.32	98.7
Water	5	4.70	..	..	4.70	100.0
Water	5	4.70	10	..	4.70	100.0
Water	5	4.70	15	..	4.69	99.7
Water	5	5.00	7.5	7.5	4.92	98.4
Urine	2	4.70	..	..	4.68	99.5
Urine	5	4.72	15	..	4.72	100.0
Urine	5	4.72	15	..	4.77	101.1
Urine	5	4.72	20	..	4.77	101.0
Urine	5	4.72	25	..	4.60	97.5
Urine[a]	2	1.90	10	1.2	1.89	99.4
Urine[a]	2	1.90	10	1.2	1.91	100.5
Urine[a]	5	1.90	10	3.0	1.90	100.0
Urine[a]	5	5.00	20	19.2	4.91	98.2
Urine[a]	5	5.00	20	19.2	4.88	97.6
Blood	4	7.60	..	..	7.62	100.2
Blood	2	3.80	..	..	3.76	99.0
Blood	1	1.90	5	..	1.88	98.9
Blood	2	0.95	10	..	0.96	101.0
Blood	1	0.47	..	..	0.47	100.0

[a] Specimen from patients after administration of 4 grams of methenamine. Urine was steam-distilled from acid solution and formaldehyde approximated colorimetrically.

Mercuric chloride, saturated aqueous solution.
Sodium hydroxide, saturated solution.
Sodium tungstate, 10 per cent solution.
Sulfuric acid, 1 *N* solution.

Procedure

The blood or urine specimen (1 or 2 cc.) is measured into the distillation tube, *F*, and the protein is precipitated by the addition of 5 cc. of 10 per cent sodium tungstate and 5 cc. of 1 *N* sulfuric acid. These quantities of tungstate and sulfuric acid for a 2-cc. blood specimen effect a complete precipitation of the proteins, and little, if any, foaming occurs during the distillation. Since urine specimens do not ordinarily contain protein, it is usually unnecessary to add the tungstate. The analyses can be made on coagulated blood; however, care must be exercised when taking a sample for the determination to obtain proportionate amounts of the serum and clot. Whenever possible, an anticoagulant, such as sodium citrate, oxalate, or fluoride, should be employed.

Ten cubic centimeters of a saturated solution of mercuric chloride and 10 cc. of a saturated solution of sodium hydroxide are measured into tube *G*. The tubes are then connected to the distillation apparatus and immersed in the hot water bath, which should be kept at the boiling temperature during the distillation process. The alcohol is steam-distilled directly into the digestion flask, *D*. After 25 to 30 cc. are distilled, 10 cc. of the 0.1 *N* potassium dichromate solution and 5 cc. of concentrated sulfuric acid are added to the distillate. The sulfuric acid should be allowed to run down the side of the flask to prevent it from mixing with the aqueous solution and causing the heat of solution to raise the temperature before the flask is closed. After the flask is closed and the cap fastened with the two springs, the solution is mixed. The flask is then placed in a boiling water bath for 20 minutes. The solution is cooled, and the dichromate solution is washed down the sides of the flask with a stream of water from a wash bottle. Enough water should be added to dilute the acid to a 10 per cent solution or less. The excess dichromate is determined by adding approximately 0.2 gram of potassium iodide crystals and titrating the liberated iodine with the standardized solution of 0.1 *N* sodium thiosulfate. Addition of the starch indicator should be delayed until the iodine color is nearly removed. Five drops of the 1 per cent starch solution are then added and the titration is completed.

Experimental

Standard alcohol solutions were made from absolute alcohol which was prepared according to the technique suggested by Noyes (*8*) and Castille and Henri (*2*). Pure acetone was prepared according to the method suggested by Frankforter and Cohen (*3*). A U. S. P. solution of formaldehyde was assumed to contain 37 per cent of formaldehyde.

Alyea and Bäckström's method (*1*) was used to test for the presence of acetone and the modified Schiff's reagent (*9*) was used to test for the presence of formaldehyde in the distillates.

Alcohol was recovered from pure solutions, blood, and urine, according to the procedure outlined above. Table I shows that quantitative recoveries of the alcohol are obtainable and that the acetone and formaldehyde are completely removed.

The efficiency of the method in removing acetone and formaldehyde was determined by distilling known quantities of these compounds and testing the distillates. It was found that the method will remove 75 mg. of formaldehyde and 30 mg. of acetone, quantities considerably in excess of those which would be present in blood or urine specimens under the most extreme conditions.

Table II shows the effect of distilling urine specimens through concentrated sodium hydroxide containing mercuric oxide. In the absence of the mercuric oxide–sodium hydroxide reagent the alcohol readings are erroneously high, especially on materials obtained from individuals receiving methenamine. On these specimens the commonly employed methods would indicate that the individual was under the influence of alcohol. While acetone does not present a very serious problem, its presence in borderline cases may increase the alcohol reading to a level considered intoxicating, especially where certain levels of alcohol in the blood or urine are being accepted for defining intoxication. It will be noted in Table II that the mercuric oxide–sodium hydroxide reagent reduces these readings to insignificant quantities.

Table II. Difference in Apparent Alcohol Reading with and without Sodium Hydroxide–Mercuric Oxide Reagent

Type of Urine	Apparent Alcohol Reading Without reagent	With reagent
	Mg./cc. of urine	
Diabetic[a]	0.115	0.036
	0.159	0.040
	0.050	0.010
	0.152	0.038
	0.060	0.010
Methenamine[b]	0.960	0.010
	0.920	0.010
	2.250	0.020
	1.770	0.030
	2.890	0.030
	1.040	0.020
	1.950	0.020
	1.060	0.030
	1.090	0.010
	1.180	0.010

[a] Diabetic patients were emergency cases, either in coma or approaching comatose condition when urine specimens were taken.
[b] Patients received 2.5 to 4.0 grams (one daily therapeutic dose) of methenamine in 8-hour period in four divided doses. Urine specimens were collected at intervals after first dose and up to 14 hours after last dose.

It has been suggested that the accumulation of intermediate metabolites in the system during various forms of anoxia may be a source of error in the alcohol determination. To test the possibility of this error, rabbits were asphyxiated in an airtight chamber until they were maximally dyspneic and cyanotic; others were rendered anoxic by exposure to carbon monoxide or to oxygen at a partial pressure of 60 mm. of mercury (equivalent to 8 per cent of oxygen at 760 mm. of mercury). These conditions failed to produce any reducing substance which would pass over with the distillate and be read as alcohol.

Summary

A method for the determination of alcohol in biological material is described. Its advantages over previously described methods are: (1) the alcohol can be separated from interfering substances, such as acids, bases, aldehydes, and ketones, in one operation and it is not necessary to test for the presence of these contaminants; (2) the distillation and digestion of the specimen are done in an all-glass apparatus, preventing contamination from stoppers. Alcohol can be recovered quantitatively when acetone or formaldehyde is present in greater concentration than would be present under the most extreme conditions. Although Table I shows that alcohol was quantitatively recovered in the presence of acetone and formaldehyde, a few samples are included in Table II to illustrate the operation of this principle on natural samples: urine from diabetic patients containing acetone bodies and from patients receiving methenamine containing formaldehyde. Accumulation of intermediate metabolites due to various types of anoxia has no effect on the alcohol reading. The method largely overcomes the criticism of nonspecificity of the methods commonly employed.

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Two Improved Pressure-Regulation Devices

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THE regulators described in this article resulted from a need for a sensitive and positive vacuum control in experiments on vapor pressures of binary mixtures.

The first instrument (Figure 1) possessed certain features and characteristics which may be useful for other purposes. No rubber tubing is necessary, for the regulator may be attached directly to the system to be controlled. Articles ordinarily found in the laboratory were used in its construction. It may be used either with weights or with the mechanism shown in Figure 2.

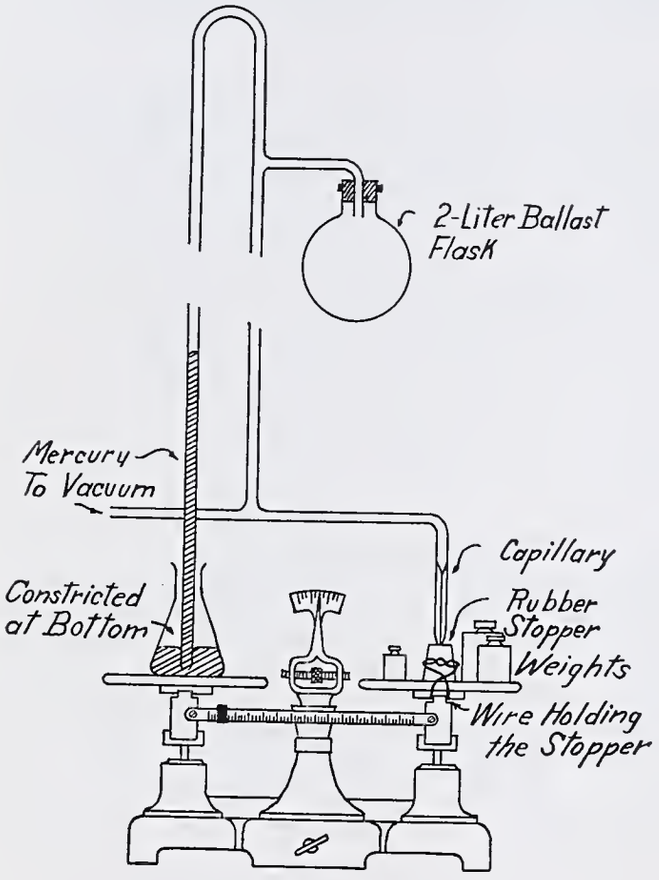

FIGURE 1

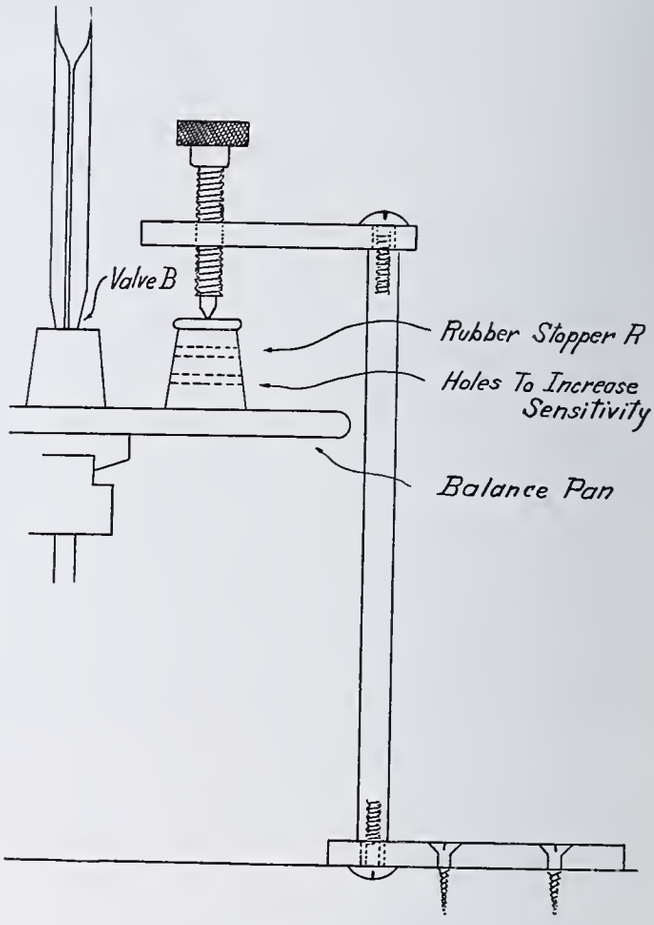

FIGURE 2

Using weights, any pressure in a range from 1 to 200 mm. is quickly obtained and kept, but above 400 mm. the pressure fluctuates and the instrument "spills", letting in large amounts of air. Control in the range from 200 to 400 mm. requires some care. The precision attainable with both regulators is 0.1 mm. in the pressure range above 100 mm. Below 100 mm. the variation in pressure is inappreciable.

The operation is shown by Figure 1. A decrease in pressure in the system decreases the force exerted on the left balance pan, indicated by the rise of the height of mercury, and permits the right-hand balance pan to lower and let air in through the capillary. The dynamic equilibrium set up in this way may be altered to raise or lower the pressure in the system by the simple addition or subtraction of weights on the right-hand pan. A table of weights corresponding to various pressures may be made, but these are subject to slight changes in the atmospheric pressure.

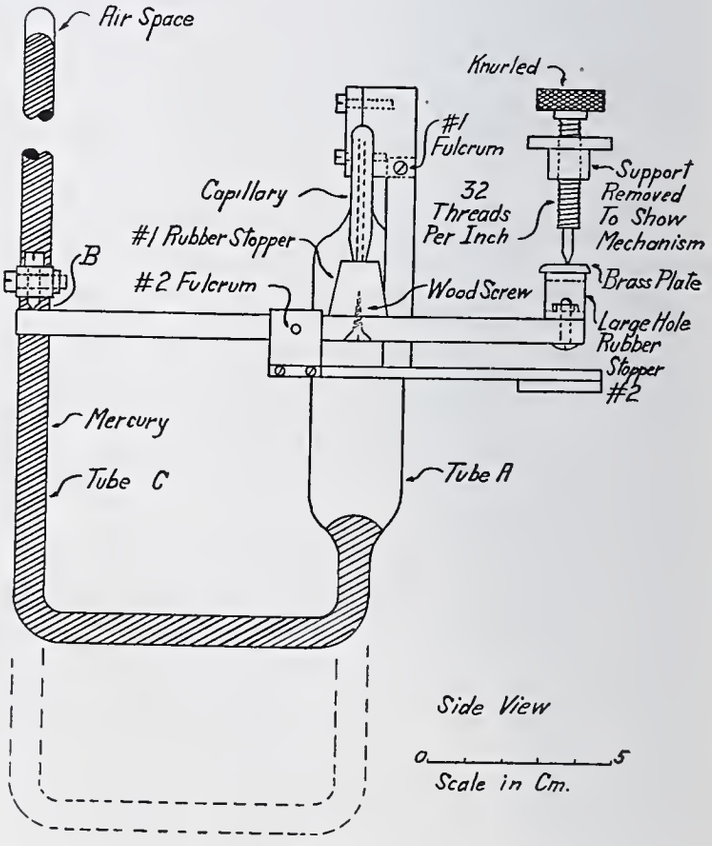

FIGURE 3

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Using the mechanism shown in Figure 2, any pressure from atmospheric down to 1 mm., with definite control at any point, may be obtained. This mechanism eliminates the disadvantages of the weights. When pressure on the balance pan is increased by turning the screw down on the perforated rubber stopper, *R*, more air is admitted to the system until the increase of air pressure in the system closes valve *B* against the pressure of *R*. Heavy sponge rubber can be used in *R*. For pressures in the system close to 760 mm. it is best not to have the source of vacuum running at full capacity.

The balance was bolted to a ring stand, which in turn was bolted to a wooden base, so that the regulator could be carried from place to place as a unit. To ensure smooth operation a ballast flask was included in the system and the bottom of the tube dipping into the mercury was constricted. About 70 ml. of mercury were used, but this could be reduced by using a smaller flask. The glass tubing was 6 mm. and the capillary was 1.5 mm. in inside diameter.

The rubber stopper used as a valve seat was an ordinary stopper boiled in sodium hydroxide solution for approximately 0.5 hour. The capillary was ground down on an emery wheel and the flat portion which presses against the rubber stopper was ground down, using first a coarse and then a fine grade of emery powder in glycerol as a grinding medium. The grinding was facilitated by clamping a cork borer in a vertical position and using it as a bearing for the capillary tube as it was rotated. In this way the plane end of the capillary is made perpendicular to the axis of the capillary.

In order to have a more compact unit and to avoid exposure of the mercury to the air, a lever arm was substituted for the balance and a closed manometer was used. An excellent instrument has been designed by Schierholtz (*1*).

Figures 3 and 4 show the construction in some detail. The screw which constitutes fulcrum 1 is not tightened up completely so that the entire glass portion moves with it as a center. As the mercury falls in the small glass arm and rises in the large portion, owing to lowering of pressure in the system, less weight is supported by the clamp pressing on the lever arm at *B* and more by fulcrum 1. The more firmly the screw mechanism presses on rubber stopper 2, the higher will the pressure rise in the system and the higher will the mercury rise in the small side arm to force rubber stopper 1 against the capillary and shut off the supply of air.

About 0.75 ml. of air left in tube *C* increases the range of pressure over which the instrument is sensitive. With the air pocket,

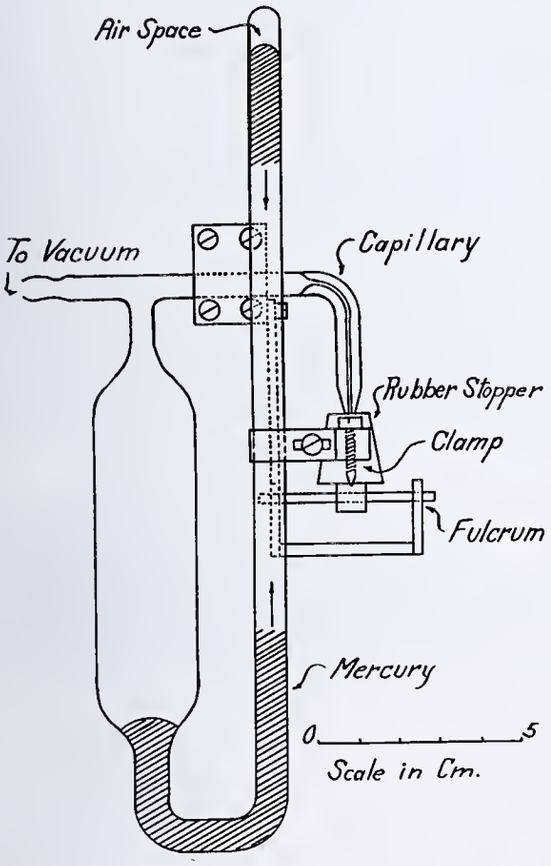

FIGURE 4

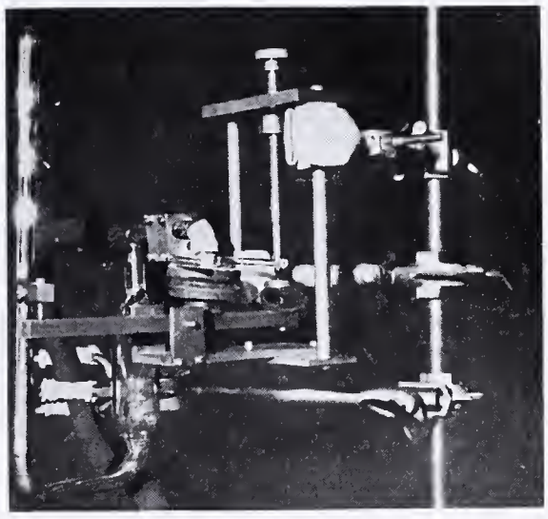

FIGURE 6

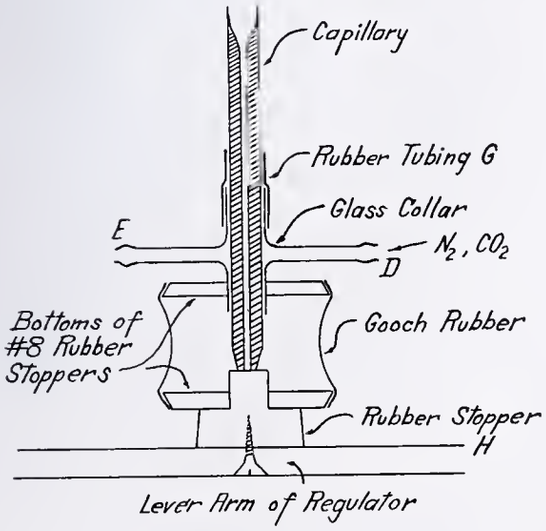

FIGURE 5

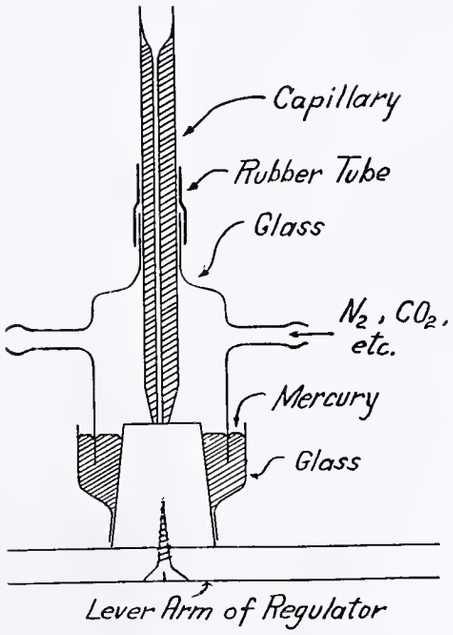

FIGURE 7

the range of control due to the movement of mercury extended from 1 to 350 mm.; without the air pocket the range was from 1 to 220 mm. Besides increasing the effective range of the regulator 100 mm., the air pocket helps to prevent the breaking of the glass arm by the mercury if the pressure in the system suddenly increases.

No rubber tubing is used in the regulator itself. Since connection to the system is made close to the fulcrum, heavy pressure tubing may be used without interfering with the action of the regulator.

If gases other than air are desired in the system during a distillation, the apparatus shown in Figures 5 and 6 may be added. This addition is facilitated by the relatively little movement between the capillary and the rubber stopper. If side arms *D* and *E* are left open to the air, air will be drawn into the system, but if nitrogen or carbon dioxide is passed through *D* and out *E*, these gases will be drawn into the system instead. Before starting a distillation the system should be flushed out with the gas to be used by applying a vacuum and having the capillary open as much as possible. The glass collar is fastened to the capillary by a short length of rubber tubing, *G*. A glass-to-glass seal is better, since no flexibility is desired at this point. Rubber stopper *H*, forming the valve seat, was cut halfway through with a cork borer and then the outside portion was cut away with a razor as shown in Figure 5. Two No. 8 rubber stoppers were bored to fit *G* and *H*, respectively, and a 4-mm. section was taken from the bottom of each. They were fastened together with a short length of Gooch rubber tubing to provide free movement between the capillary and rubber valve seat.

In Figure 7 the same effect is accomplished using a mercury seal. This is much more rugged and easily handled, but a sudden increase of nitrogen or carbon dioxide pressure might blow out the mercury.

Acknowledgment

The author wishes to acknowledge the helpful encouragement of P. A. van der Meulen and D. L. Cottle. Appreciation is also due F. G. Horstmann, Belleville, N. J., for the unlimited use of his machine shop.

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Disposal of Acid Fumes in Wet Assaying

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WORKERS in old or improvised laboratories are frequently confronted with the problem of disposal of acid fumes from wet-assay digestions. If the fume-laden gases are passed through zeolite sand covered with water, the fumes are effectively removed. The surface afforded by the grains of sodium aluminum silicate adsorbs and reacts with the fumes rapidly and completely and allows relatively large volumes of inert gas to be passed without the passage of the acid fumes. The fumes are removed so completely that any type of evacuating pump can be employed with the exhaust open to the laboratory.

The commercial grade of synthetic zeolite sand gradually dissolves and passes off with the condensed overflow. The absorbent is most economical.

The application of this procedure to micro-Kjeldahl digestion is illustrated in Figure 1.

Bottle *B* contains zeolite sand covered with water. *A* acts as a trap for sand grains from *B*. A spray trap, *C*, employing the indentations featured in the Vigreux type of fractionating head, drains well and efficiently breaks up the spray. The tip, *D*, drains the side arm and prevents the formation of fluid locks in the system, which would cause fumes to escape at the open connections. A trap, *C*, fits into the mouth of each digestion tube or flask and serves to prevent loss of sample in the spray and to connect the digestion vessel to the fume-absorbing system.

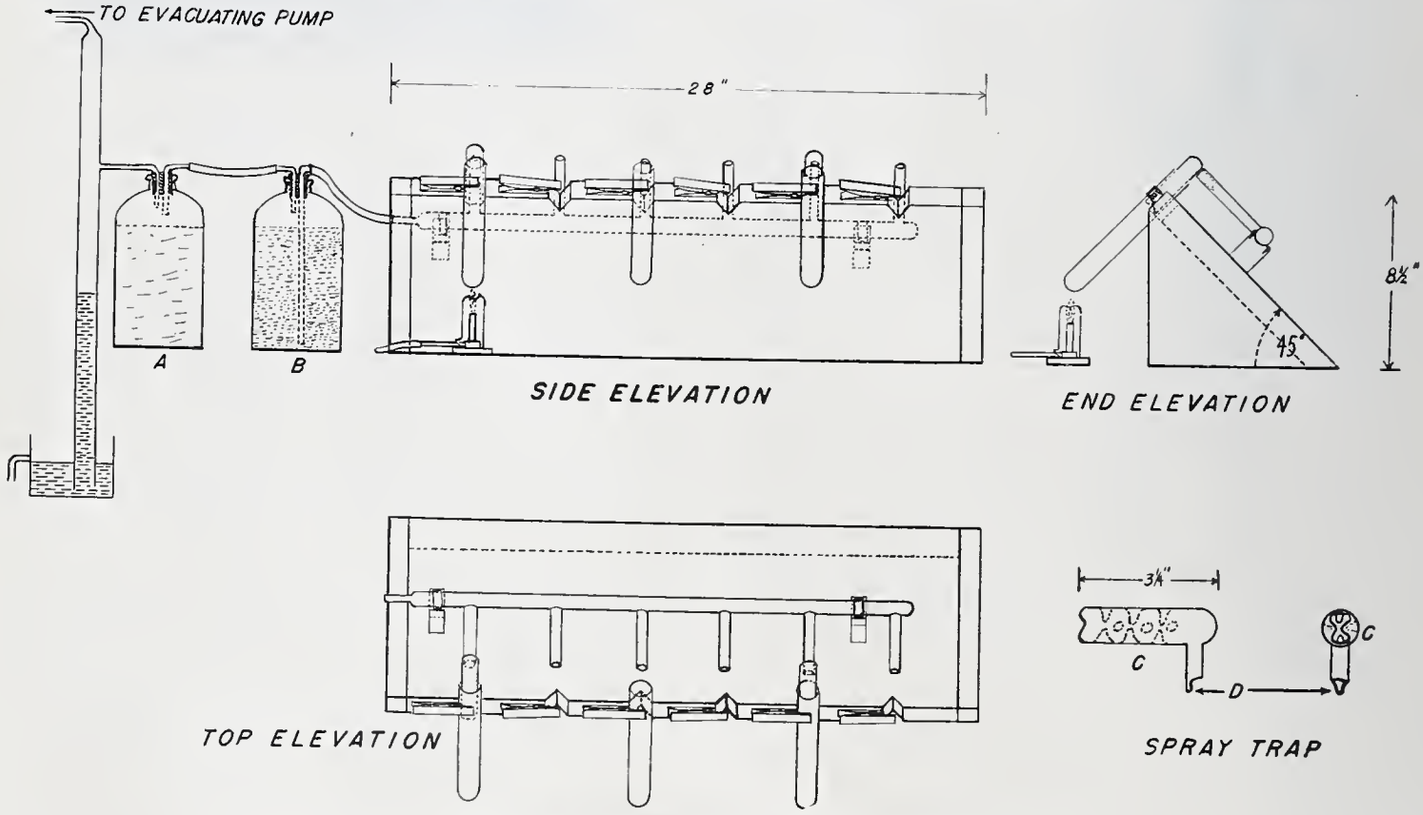

FIGURE 1. DIGESTION RACK FOR MICRO-KJELDAHL DETERMINATIONS

A Reaction Tube for Determination of 17-Ketosteroids

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IN THE method of Holtorff and Koch (*2*) for the determination of 17-ketosteroids, the presence of more than a trace of carbonate in the reaction mixture results in turbidity in the final diluted alcoholic solution. Absorption of carbon dioxide during the reaction may be prevented by use of the tube shown in the accompanying figure, in which the concentrated alkaline solution is protected from contact with the air in the small space at the bottom of the tube below the long-handled stopper.

To avoid wetting the ground joint between the stopper and the shoulder of the tube, with resulting exposure of part of the solution, the 0.2-ml. portions of alcoholic extract, *m*-dinitrobenzene, and aqueous potassium hydroxide are delivered onto the top of a short glass rod fused into the bottom of the tube. The spiral form of this rod aids in rapid thorough mixing with the diluent later. When sufficient time has elapsed for completion of the reaction, 10 ml. of alcohol are pipetted into the tube above the stopper. From a series of tubes the stoppers may be removed in rapid succession, thus decreasing the interval between dilutions to more nearly that required in making the readings. The concentrated solution and the diluent alcohol are mixed by a rapid swirling motion, and are transferred to the Evelyn colorimeter tubes as required. By the use of this reaction tube, long contact of strongly alkaline solutions with the colorimeter tubes is avoided.

The small bottom part is of 15-mm. tubing (outside diameter) and has a depth below the ground joint of approximately 20 mm. The upper part is of 25-mm. tubing of any convenient length (an over-all length of 115 mm. was used). The stopper is easily made of 1-mm. capillary tubing which gives it sufficient weight to sink in bichromate–sulfuric acid cleaning solution. The stopper is ground in with a simultaneously lateral and rotary motion of the handle to produce spherical ground surfaces which fit in any position of the handle.

In this laboratory, the potassium hydroxide solution is stored in and delivered from a small mercury-sealed buret (*1*, Figure 3) having a delivery tip sufficiently long to reach to the top of the spiral glass rod. The tip of the Koch buret (*3*) is also long enough for this purpose. Many ordinary burets require an extension of the tip, preferably fused on.

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Sample Carrier for Organic Liquids

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IN THE ultimate analysis of organic liquids the common sample carriers are sealed bulblets or open porcelain boats, the sealed bulblets for use with low-boiling liquids and the open boats for use with high-boiling liquids.

A carrier device suggested for use with liquids of intermediate boiling temperatures does away at once with the time consumed when sealed bulblets are used and the loss by evaporation when open boats are used. Figure 1 shows the device suggested.

Using a piece of Pyrex tubing approximately 6 × 30 mm., the container is prepared as follows: One end is melted completely, blown slightly, and pressed firmly onto a flat, noncombustible surface. The stem is given a curvature and the open end is constricted by rotating in a flame until the opening is approximately 1 mm. in diameter.

The container is filled by means of an eye dropper with a capillary tube, and may be used over and over again. The flat bottom makes it possible to stand the object directly upon the balance pan.

For the actual combustion, the container is put into a porcelain boat with the opening downward and toward the end of the boat nearer the catalyst (Figure 1, right). The curve in the neck keeps the liquid in the container until it is distilled out. Small pieces of glass in the container aid in the regular distillation of the sample into the boat. The speeds of distillation and combustion are thus controlled.

Just as some liquids do not volatilize completely when distilled from a bulblet carrier, so some liquids will leave a small carbonaceous deposit when distilled from the suggested

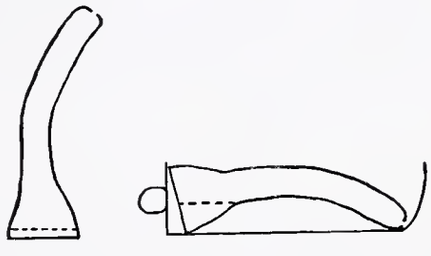

FIGURE 1

carrier. This difficulty may be partly overcome by decreasing the speed of distillation. However, if a small deposit does occur, it may be completely removed by heating the carrier to a dull red temperature for a few minutes while the oxygen flow is continued. Nevertheless this device will undoubtedly be limited, for practical use, to liquids which char very little or not at all when distilled.

The author has obtained very satisfactory results with liquids of intermediate boiling temperatures.

AIDED by a grant from the Illinois Academy of Science, and certain apparatus loaned by Dr. Lauder of the Pet Milk Laboratory.

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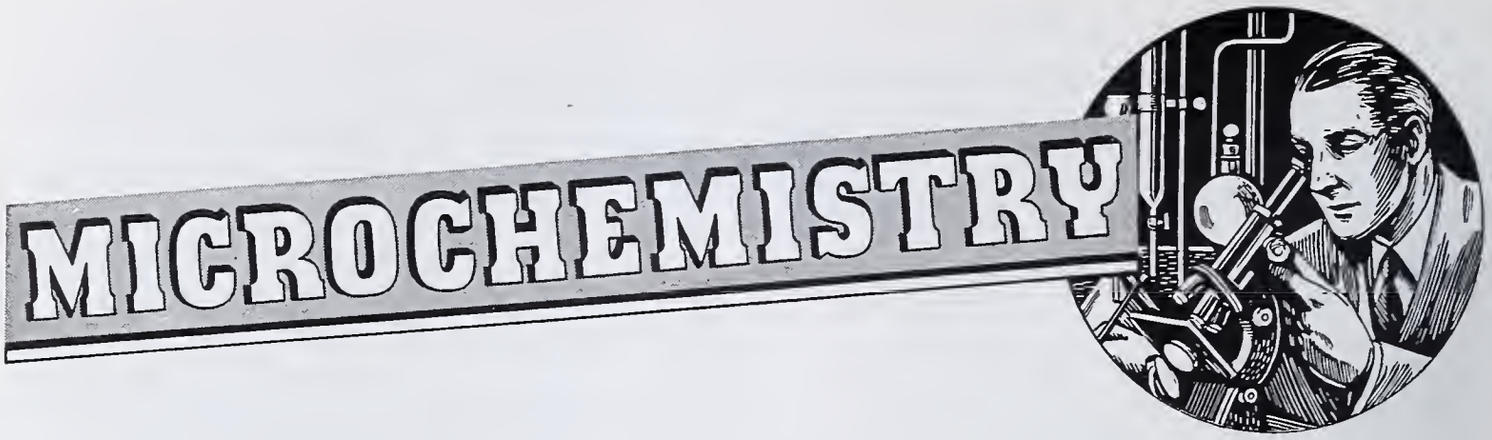

Iodobismuthite Determination of Bismuth in Biological Samples

Application of Neutral Wedge Photometer

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George A. Breon & Co., Inc., Kansas City, Mo.

THE estimation of bismuth by the iodobismuthite method was originated by Thresh in 1880 (*6*). Application of this method to the determination of bismuth in biological samples was attempted by various investigators and their efforts were consummated in a method published by Leonard (*3*) in 1926. Several modifications (*1*, *2*) of the Leonard procedure have been employed since that time. A review of the several methods and their modifications has been compiled by von Oettingen (*4*).

In the early nephelometric methods it was necessary to employ small quantities of reagents and in most cases to employ the entire analytical sample in making a determination, so that any error due to faulty manipulation in the color developing stage could not be readily corrected. The precision of the determinations was limited by the visual interpolation of the operator.

The method which has been developed for use with a neutral wedge photometer overcomes these difficulties. This method is applicable to the estimation of quantities of bismuth of the order of 50 to 500 micrograms in any solution which has been freed of interfering substances. With proper modifications as little as 10 micrograms could be determined accurately, since samples containing only 3 micrograms of the element in 15 cc. are needed.

Reagents

STANDARD BISMUTH SOLUTION. Dissolve 1.0000 gram of freshly fractured and pulverized bismuth in 5 to 10 cc. of concentrated nitric acid in a covered casserole. When solution is complete dilute with 100 cc. of distilled water. Wash the cover glass thoroughly and add the washings to the solution in the casserole. Transfer the solution quantitatively to a 1-liter volumetric flask and dilute to the mark with distilled water. This solution contains 1.00 mg. of bismuth per cc.

SULFURIC ACID, 20 PER CENT. Slowly add 200 grams of concentrated sulfuric acid to 800 grams of distilled water.

POTASSIUM IODIDE, 5 PER CENT. Dissolve 50 grams of potassium iodide (U. S. P.) in 950 grams of distilled water.

HYPOPHOSPHOROUS ACID, 10 PER CENT. Dilute 200 grams of hypophosphorous acid (purified, 50 per cent) with 800 grams of distilled water.

Method

The yellow potassium iodobismuthite solution which constitutes the basis of the method is stated by Rasmussen, Jackerott, and Schou (*5*) to absorb light up to 4500 Å. They employed the mercury line at 4359 Å. in a photometric investigation of the method of Leonard.

To determine the most satisfactory wave band for use in the determinations with the neutral wedge photometer, absorption curves for solutions containing different concentrations of bismuth were made. The data appearing in Table I and plotted in Figure 1 show that concentrations of bismuth up to 300 micrograms per 50 cc. can be effectively determined, using a 100-mm. cell and a filter transmitting light of 460-millimicron wave length.

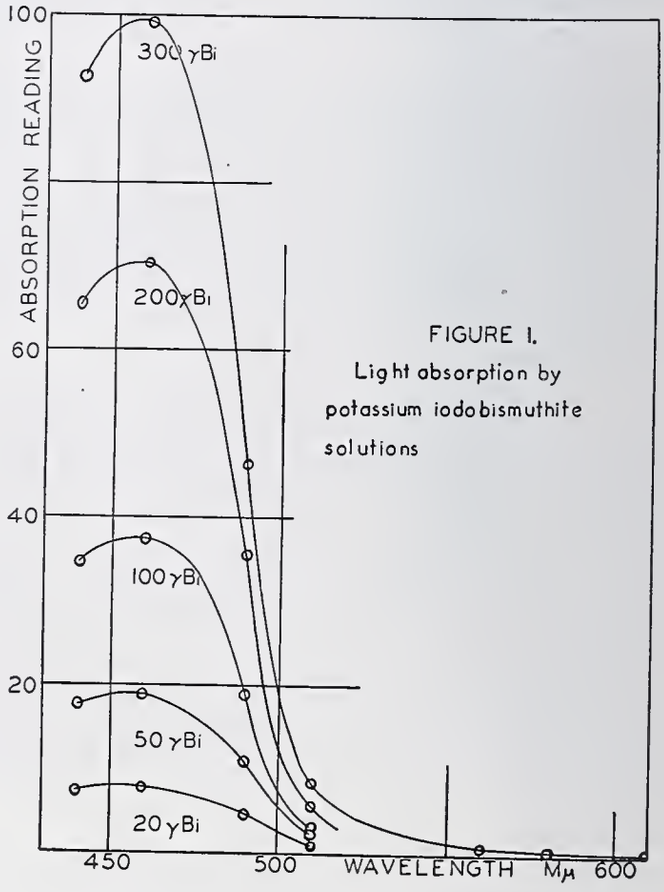


FIGURE 1.
Light absorption by potassium iodobismuthite solutions

Table I. Absorption Data for Potassium Iodobismuthite Solutions[a]

Bismuth	Absorption Readings at Wave Length[b]: 440	460	490	510	560	580	610
Micrograms/ 50 cc.							
20	7.5	7.6	4.7	2.3	0.5	0.3	0.2
50	17.6	18.4	10.6	2.5	0.7	0.3	0.2
100	34.5	37.3	18.8	2.7	0.4	0.3	0.2
200	65.2	70.5	35.5	5.5	0.3	0.5	0.2
300	92.5	99.3	46.2	8.5	0.3	0.4	0.2

[a] Using 100-mm. absorption tube.
[b] Effective wave length in millimicrons of eyepiece filters supplied by American Instrument Co.

Adding 10 cc. of 5 per cent potassium iodide and 10 cc. of 10 per cent hypophosphorous acid to a sulfuric acid solution of bismuth and making up to 50-cc. volume with water provide a satisfactory medium for conducting the estimation. Since the instrument cell has a volume of about 15 cc., not all of this solution is needed; part of it provides a rinse for the cell before making the reading. These solutions may be prepared in any 50-cc. volumetric container.

The data in Table II, which have been plotted in Figure 2, present the evidence for the choice of the above components of the solution. Potassium sulfate and ammonium sulfate have been included in the experiment because they are usually present in samples prepared by the digestion of organic materials. Inspection of the data shows that only the potassium iodide affects the color intensity to any appreciable extent. Therefore it is the only reagent included in Figure 2.

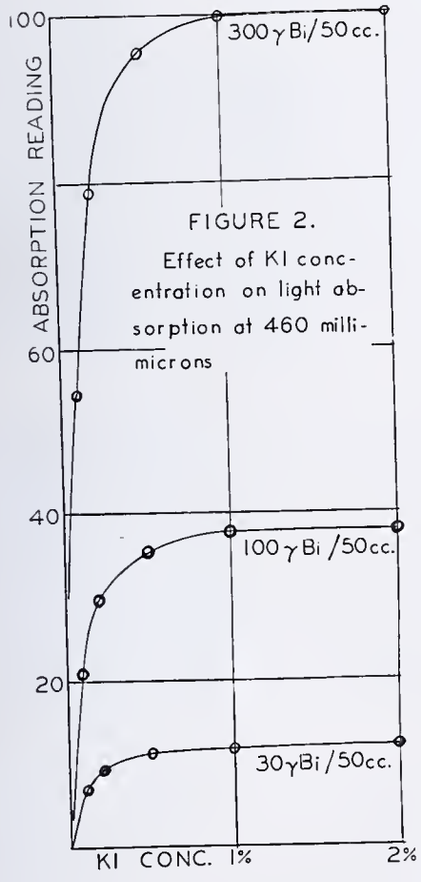


FIGURE 2. Effect of KI concentration on light absorption at 460 millimicrons

The choice of hypophosphorous acid was made after the sulfites and sulfurous acid which are usually employed as reducing agents were found to contain an impurity that developed an interfering yellow color with potassium iodide in acid solution. Samples from different manufacturers showed the presence of the same impurity. Sulfurous acid prepared from sodium thiosulfate crystals also produced an interfering color. It was found that both photographic glycin (*p*-hydroxyphenyl aminoacetic acid) and hypophosphorous acid were free of such impurities. Glycin was abandoned because it develops an interfering color in the presence of ferric iron. Hypophosphorous acid proved suitable in every respect.

The calibration curve for the instrument was made by determining the absorption at 460 millimicrons with known quantities of bismuth. A blank solution of the reagents was used to establish the zero point of the instrument. These data are shown in Table III and Figure 3. The slight deviation from Beer's law apparent in the curves is probably due to the potassium iodide–bismuth ratio in the solution. The deviation is not of sufficient magnitude to warrant an increase in the potassium iodide content in the solution from which color absorption readings are to be made.

Estimation of Bismuth in Urine

For the estimation of bismuth in biological samples the organic matter is first destroyed by wet-digestion of the sample in a sulfuric acid mixture. The ultimate product is a solution of bismuth sulfate in 100 cc. of dilute sulfuric acid. Aliquot portions of this solution are taken for the preparation of the iodobismuthite solution from which the final estimation is made.

The presence of small quantities of elements other than bismuth in the prepared urine samples causes considerable

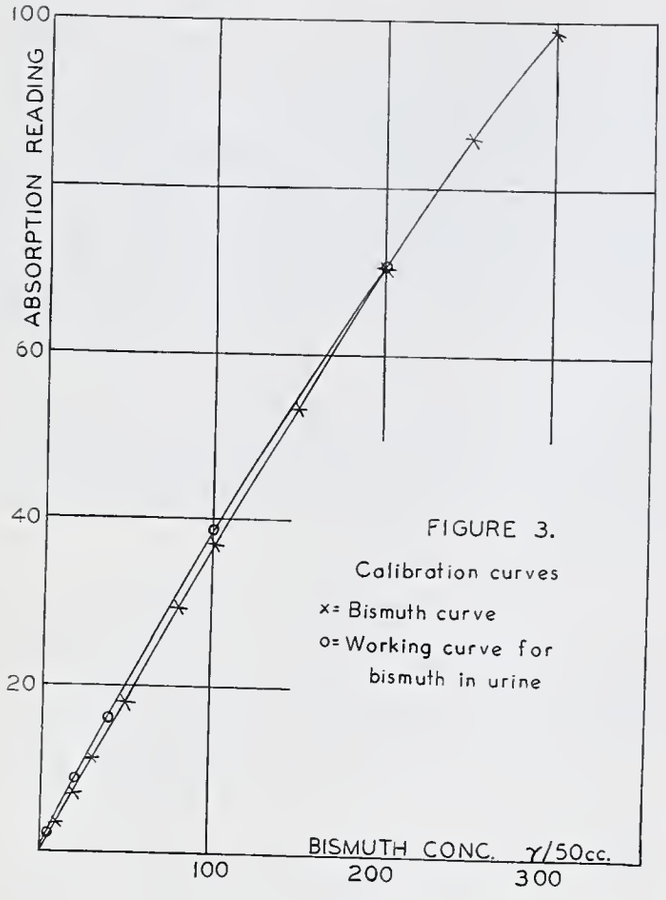
Table II. Effect of Solution Components on Development of Potassium Iodobismuthite Color

Bi Added	20% H_2SO_4	5% KI	10% H_3PO_2	10% K_2SO_4	10% $(NH_4)_2SO_4$	Absorption Reading for 100-Mm. Tube at 460 Millimicrons
Micrograms	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	
300	10	1	10	..	..	54.2
300	10	2	10	..	..	78.6
300	10	5	10	..	..	95.5
300	10	10	10	..	..	99.9
300	10	20	10	..	..	100.0
100	10	1	10	..	..	20.7
100	10	2	10	..	..	29.6
100	10	5	10	..	..	35.2
100	10	10	10	..	..	37.7
100	10	20	10	..	..	37.7
30	10	1	10	..	..	6.7
30	10	2	10	..	..	9.4
30	10	5	10	..	..	11.3
30	10	10	10	..	..	11.9
30	10	20	10	..	..	12.0
100	2	10	10	..	..	36.4
100	20	10	10	..	..	38.5
100	10	10	10	..	..	39.6
100	10	10	5	..	..	38.3
100	10	10	20	..	..	37.5
100	10	10	10	5	..	38.7
100	10	10	10	20	..	38.8
100	10	10	10	..	5	38.2
100	10	10	10	..	20	38.8

Table III. Bismuth Calibration Curve

(100-mm. absorption cell with 460-millimicron filter. Solutions made to contain in 50 cc., 10 cc. of 20% H_2SO_4, 10 cc. of 5% KI, and 10 cc. of 10% H_3PO_2)

Bismuth Added	Absorption Reading
Micrograms	
10	3.6
20	7.2
30	11.4
50	17.9
80	29.2
100	36.9
150	53.4
200	70.1
250	86.0
300	98.9



error when determinations are made in the lower range. This is due to the deviation of the curve from the bismuth calibration curve (Figure 3). However, by establishing a working curve for urine samples using samples containing known amounts of bismuth (Table IV, Figure 3) determinations can be made with less than 5 per cent error in the range of 20 to 3000 micrograms of bismuth per sample (Table V). Operating similarly, the method may be employed for tissue analysis. Some selected results are shown in Table VI.

Procedure

Place 100 cc. of urine in a 500-cc. Kjeldahl flask with 10 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate, and 25 cc. of concentrated nitric acid. Add two glass beads and concentrate the urine to a small volume by rapid boiling. Digestion of the organic matter follows and proceeds smoothly. When the solution is clear and usually colorless (about 1 hour) allow it to cool and add 40 to 50 cc. water. Filter the solution into a 100-cc. volumetric flask, wash the Kjeldahl flask and filter with successive small portions of water, and add the washings to the main portion of the filtrate. Finally adjust the volume to the mark with water and mix well. This constitutes the prepared sample from which the bismuth determination is made.

Table IV. Calibration Curve for Bismuth in Urine

(100-mm. absorption cell with 460-millimicron filter. Solutions made to contain in 50 cc. 10 cc. of 5% KI, 10 cc. of 10% H_3PO_2, and stated quantity of 100-cc. prepared samples. 100 cc. of urine digested)

Bismuth Added	Aliquot	Bismuth in Aliquot	Absorption Reading				Deviation from Mean
			I	II	III	Av.	
Mg.	*Cc.*	*Micrograms*					%
0.02	20	4	..	2.0	2.4	2.2	10
0.10	20	20	8.9	8.8	8.3	8.7	5
0.2	20	40	15.9	16.6	15.5	16.0	3
0.5	20	100	38.8	38.5	38.5	38.6	1
1.0	20	200	70.5	70.3	70.4	70.4	0.2
2.0	10	200	70.2	70.3	69.8	70.1	0.4

Pipet an aliquot portion (20 cc.) of the prepared sample into a 50-cc. volumetric flask and add 10 cc. of 5 per cent potassium iodide from a delivery pipet. Mix the two by shaking. To this add 10 cc. of 10 per cent hypophosphorous acid and sufficient distilled water to make up the volume. Mix the sample and rinse the absorption cell with a portion of it. Fill the cell with the solution and wipe the polished ends with a clean dry cloth before making the absorption reading. The micrograms of bismuth corresponding to the absorption readings are read off from the working curve and the bismuth content of the prepared sample is calculated.

The above sequence of reagent addition must be followed because potassium iodide is oxidized to iodine by ferric iron and other oxidizing impurities. This is capable of reduction by hypophosphorous acid, whereas the original oxidizing agents are not. This procedure yields a solution which does not change in color for several hours.

Table V. Determination of Bismuth in Urine

Bismuth Added to 100 Cc. of Urine	Absorption Reading	Bismuth Found	Error
Mg.		*Mg.*	%
0.02	2.0	0.02	0
	2.0	0.02	0
	2.3	0.02	0
0.10	8.5	0.10	0
	8.1	0.095	5
	8.1	0.095	5
0.20	16.2	0.20	0
	16.2	0.20	0
	16.4	0.21	5
0.50	37.0	0.48	4
	37.3	0.49	2
	37.2	0.49	2
1.0	71.3	1.01	1
	71.5	1.01	1
	71.4	1.01	1
2.0[a]	69.8	1.98	1
	69.0	1.95	3
	69.3	1.96	2
3.0[a]	98.0	2.98	0
	98.2	2.98	0
	98.5	2.99	0

[a] 10-cc. portion of the 100-cc. prepared sample employed instead of usual 20-cc. portion.

Table VI. Typical Tissue Analyses

Tissue	Weight	Bismuth Added	Bismuth Found	Error
	Grams	*Mg.*	*Mg.*	%
Liver	75	0.1	0.10	0
Muscle	75	0.5	0.50	0
	75	0.5	0.49	2
	75	1.0	1.01	1
	75	1.0	1.01	1
	75	4.0	3.96	1
	75	4.0	3.96	1

Summary

The concentration of bismuth in biological samples by the iodobismuthite method may be rapidly and accurately determined with the neutral wedge photometer. The method is not recommended when less than 20 micrograms of the element are available in the analytical sample. For analyses of various materials, working curves may be prepared and employed, as in the example for bismuth in urine.

The major factor in analyses by this method is the concentration of potassium iodide. This factor has not been previously considered of critical importance and different investigators have employed varying concentrations of this reagent in developing the iodobismuthite color. The effect of small variations in the volume of 5 per cent potassium iodide solu-

tion is shown in Figure 2. In determining bismuth in the range 20 to 3000 micrograms, the concentration of potassium iodide should be at least 1 per cent. The Leonard method employs about 0.3 per cent potassium iodide. In this range of potassium iodide concentration a small error in measurement of the potassium iodide could result in an appreciable error in the bismuth content. The technique here described, if followed precisely, will result in the minimum error due to measurement of reagents.

This method has been employed routinely in the investigation of the bismuth content of biological samples in conjunction with studies on the absorption and excretion of bismuth by the animal organism originating in these laboratories (*7*).

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Determination of Arsenic in Biological Material

A Photometric Method

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STUDIES necessitating the accurate quantitative determination of arsenic in biological material made apparent the need for a rapid and flexible method of analysis by which large numbers of samples, containing arsenic in amounts ranging from less than a microgram to several milligrams, could be handled simultaneously. Recent literature describes a number of methods (*1*, *2*, *4*, *5*, *6*) of acceptable accuracy but none of them filled the author's needs completely. Combination of the better features of these methods plus some slight modifications resulted in the development of a procedure which met all requirements. This modified method consists essentially of the wet-ashing procedure suggested by Kahane and Pourtoy (*4*) and later used by Morris and Calvery (*6*), the evolution of arsenic trichloride from the prepared sample in the manner described by Scherrer (*8*) and Rodden (*7*), and a final photometric measurement similar to that described by Morris and Calvery (*6*).

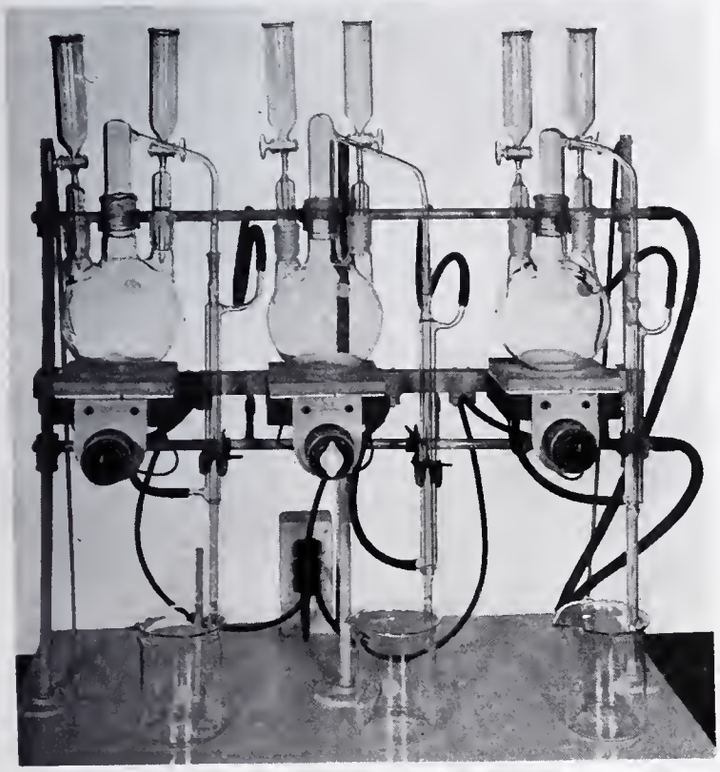

FIGURE 1. WET-ASHING APPARATUS

Reagents and Apparatus

High-grade chemicals are used throughout the analysis to ensure a low arsenic blank. Grasselli C. P. reagent nitric, sulfuric, and hydrochloric, and Mallinckrodt analytical reagent perchloric and hydrobromic acids and ammonium molybdate have been found suitable.

Before use all glassware (Pyrex) is washed thoroughly with hot dilute nitric acid (50 ml. of nitric acid, specific gravity 1.42, per 100 ml.) and rinsed with distilled water to remove arsenic present as surface contamination from previous use.

The photoelectric spectrophotometer, previously described (*3*), is employed for density measurements. Readings are taken with the monochromator set at 620 mμ. Aminco, Style D, class 3, high-precision matched cells, holding approximately 8 ml. and with an internal length of 100 mm., are used.

Samples are wet-ashed in 1-liter distilling flasks provided with three necks carrying ⅘ interchangeable ground-glass connections, into which are fitted the distilling head and two separatory funnels, as described by Morris and Calvery (*6*). Heat is furnished by electric heaters connected with Variacs for voltage control and subsequent temperature regulation. The essential portions of the complete setup are illustrated in Figure 1, which also shows the stand holding a battery of three flasks.

The apparatus for isolation of arsenic from the ashed sample is shown in Figure 2. Two units were found to be sufficient. The device is similar to that described by Scherrer (*8*) and Rodden (*7*), but has been modified by the introduction of three ⅘ interchangeable ground-glass connections which greatly facilitate charging and cleaning the apparatus. The thermometer used (range 0° to 250° C.) has a 7.5-cm. (3-inch) stem below the ⅘ joint which fits into a female connection in the side of the flask. No trouble has been encountered with sticking joints, especially if the apparatus is dismantled while warm. The distilling head is connected with a downward water-cooled Allihn condenser, the lower end of which dips below the surface of the distilled water contained in a tall cylinder surrounded by an ice-water bath. A Cenco adjustable electric heater is used as the source of heat.

Procedure

ASHING THE SAMPLE. Place the sample (100 ml. or less of urine, or 10 grams or less of other biological material such as blood or tissue) in the 1-liter distillation flask (Figure 1). Add 20

ml. of concentrated sulfuric acid (specific gravity 1.84), 20 ml. of concentrated nitric acid (specific gravity 1.42), and several pieces of Carborundum, and mix well. Then insert the flask with contents, connect with the apparatus, and gently heat. One separatory funnel contains 5 ml. of perchloric acid (70 to 72 per cent) and the other, 30 ml. of concentrated nitric acid (specific gravity 1.42). Raise the temperature by Variac adjustment just to boiling and start the addition of the nitric acid drop by drop, catching the distillate in an open beaker. When approximately 15 ml. have been added, the major portion of the organic matter will have been oxidized, and the color of the solution will have become lighter; at this point, add the perchloric acid, drop by drop, until the entire 5 ml. have been introduced; meanwhile continue to add the remainder of the nitric acid, reserving 1 ml. in the funnel. Continue heating until dense white fumes of perchloric acid begin to appear. If charring occurs at this point, dispel by drop-by-drop addition of the remaining 1 ml. of nitric acid. In any case, add the remainder of the nitric acid and continue heating for 30 minutes after the initial formation of copious fumes of sulfur trioxide. (Excessive charring should be avoided by the immediate addition of nitric acid when charring occurs. Excess perchloric acid in the presence of sulfuric acid as used here has not been found dangerous.) Allow the distillation flask with contents to cool, disconnect, and transfer the entire contents to a 50-ml. glass-stoppered cylinder, rinsing several times with small portions of distilled water. Mix well and when cool dilute to the mark at 20° C.

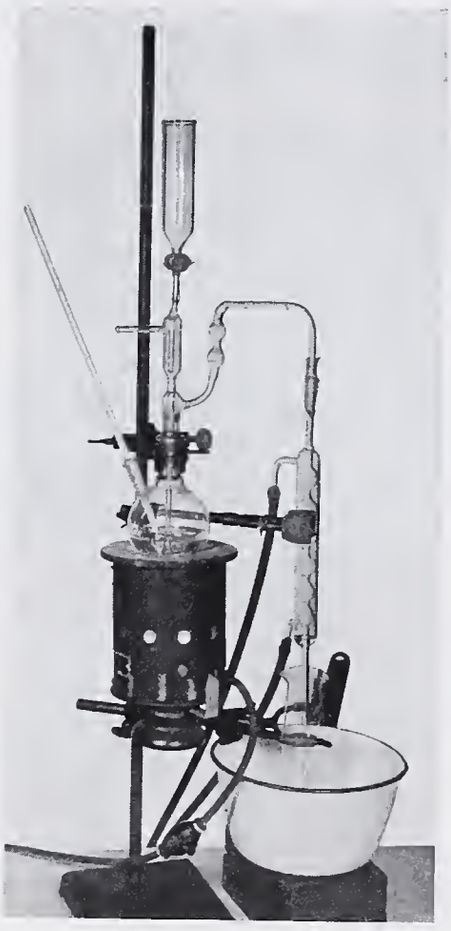

FIGURE 2. DISTILLATION APPARATUS

ISOLATION OF ARSENIC AS TRICHLORIDE. Transfer one half of the prepared sample (25 ml.) by means of the separatory funnel to the distillation flask (200-ml. capacity) used for volatilization (Figure 2). In the same manner add 5 ml. of hydrobromic acid (40 per cent) and 40 ml. of concentrated hydrochloric acid (specific gravity 1.19). Mix 1 gram of hydrazine sulfate with 30 ml. of concentrated hydrochloric acid (specific gravity 1.19), crushing lumps with the flattened end of a glass stirring rod, and add this also through the separatory funnel, sealing the stopcock with a few milliliters of distilled water. Connect a carbon dioxide cylinder to the side opening and allow gas to bubble through the liquid in the distillation flask at a rate of approximately 4 bubbles per second. Heat by means of the Cenco adjustable heater and allow the temperature to rise gradually to 111° C., catching the distillate in 40 ml. of distilled water in a tall glass cylinder immersed in ice water (about 20 ml. of distillate come over during a heating period of approximately 35 minutes). Transfer the distillate plus a few milliliters of distilled water used to rinse down the condenser walls to a 100-ml. flask containing 25 ml. of concentrated nitric acid (specific gravity 1.42) and make up the volume to the mark with distilled water. Transfer all or a suitable aliquot to a 100-ml. beaker, cover with a Fisher Speedyvap beaker cover, and evaporate to dryness. Remove beaker cover and heat the beaker with contents in an electric oven at approximately 120° C. for one hour to remove all trace of oxidizing agents.

DEVELOPMENT OF COLOR. Add the correct amount of developing solution, as indicated in Table I, to the beaker containing the dried residue; cover with a cover glass and place in a water bath (temperature maintained at 70° to 75° C.), until the development of the blue color is complete—generally 30 minutes suffice but 45 minutes will not harm the developed color. [The developing solution contains 10 per cent by volume of acid molybdate solution (1 gram of ammonium molybdate per 100 ml. of 5 *N* sulfuric acid) not more than one week old, 89 per cent by volume of distilled water, and 1 per cent by volume of a freshly prepared aqueous hydrazine sulfate solution (0.15 gram per 100 ml.), added and mixed together in the order given. The solution should be mixed in necessary amounts as used.] When the color has developed to its full intensity, remove the beaker with contents from the water bath, allow to cool, and transfer quantitatively to the proper-sized graduated cylinder (Table I), mixing well and diluting to the mark at 20° C.

PHOTOMETRIC MEASUREMENT OF COLOR DENSITY. Density measurements may be made immediately or 24 hours later. The same pair of cells is used for all ranges, one containing the solvent (distilled water), and the other the solution. The readings are evaluated from working curves obtained with known amounts of arsenic (as an aqueous solution of arsenic pentoxide) treated in the same way as the evaporated and dried distillate residues.

TABLE I. ARSENIC RANGE, VOLUMES OF DEVELOPING SOLUTION, AND FINAL DILUTIONS OF DEVELOPED COLOR

Range *Micrograms*	Volume Used *Ml.*	Final Dilution *Ml.*
0– 10	5	10
0– 50	25	50
0–100	50	100

TABLE II. RECOVERY OF KNOWN AMOUNTS OF ARSENIC ADDED TO URINE

(50-ml. samples)

Range Used *Micrograms*	Arsenic Added *Micrograms*	Arsenic Found *Micrograms*	Arsenic Recovered *Micrograms*
0– 10	Nil	2.0[a]	....
0– 10	Nil	2.1[a]	....
0– 10	Nil	3.9	....
0– 10	Nil	3.9	....
0– 10	1.0	4.9	1.0
0– 10	1.0	5.1	1.2
0– 10	5.0	9.0	5.1
0– 10	5.0	9.1	5.2
0– 50	10.0	14.0	10.0
0– 50	10.0	14.5	10.5
0– 50	15.0	19.5	15.5
0– 50	15.0	19.0	15.0
0– 50	25.0	29.0	25.0
0– 50	25.0	28.5	24.5
0– 50	50.0	53.5	49.5
0– 50	50.0	53.5	49.5
0–100	75.0	79	75
0–100	75.0	78	74
0–100	100.0	103	99
0–100	100.0	104	100
0–100	2000.0	1980	1980
0–100	2000.0	1980	1980

[a] Calculated as a reagent blank, 50 ml. of distilled water being substituted for 50 ml. of urine.

Analytical Results

In Table II are listed results obtained by the analysis (in duplicate) of 50-ml. samples of urine containing known added amounts of arsenic, and in Table III, results from 5-gram samples of whole blood. The first two results in the "Arsenic Found" column in each table represent the actual reagent blanks obtained by substituting equivalent amounts of dis-

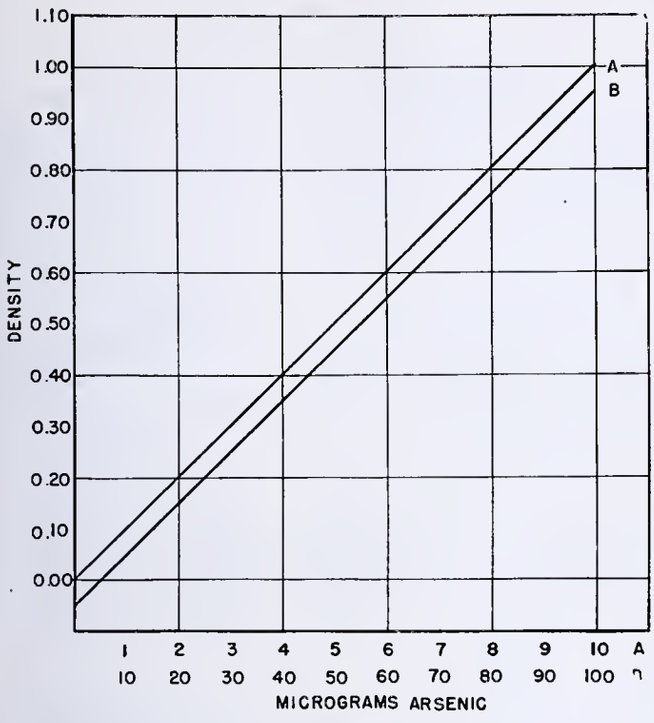

FIGURE 3. ARSENIC CURVE

tilled water for 50 ml. of urine and for 5 grams of blood, respectively.

Discussion

The size of the aliquots of prepared samples chosen for distillation and color development is dictated by experience. In most cases one half of the prepared sample may be used if the quantity of arsenic present in the aliquot is not greater than 1 mg., the maximum quantity ordinarily distilled under the conditions outlined above. Aliquots of the distillates also may be used for color development, but the amount of arsenic in the aliquot should not exceed 100 micrograms. If more than this amount is present, the color development should be repeated with a smaller aliquot of the distillate; if this is not possible, the distillation step must be repeated with a smaller portion of ashed sample. In the latter event, care must be taken to see that 10 ml. of sulfuric acid (specific gravity 1.84) are present during the distillation. In the case of certain materials, particularly blood and spinal fluid, usually low in arsenic, and when only very small samples are available, the entire sample is used for the analysis.

The technique of wet-ashing employed reduces to a minimum the amount of arsenic lost by entrainment in the distillate, in confirmation of Morris and Calvery's (*6*) work. A definite excess of nitric and perchloric acids is maintained at all times during the destruction of organic matter, and entrainment losses are negligible as compared with the sensitivity of the final photometric measurement. A separate analysis of the distillate from the wet-ashing of 100 ml. of urine containing 4000 micrograms of arsenic showed a loss of only 12 micrograms or 0.3 per cent of the original content.

The blue color produced in the conversion of arsenic acid to an arsenic-molybdenum compound by treatment with a solution containing hydrazine sulfate and ammonium molybdate has been found to be very stable; density measurements have remained constant over a period of 24 hours, obeying Beer's law; this confirms the findings of Rodden (*7*). The temperature of the water bath used in color development may range from 70° to 75° C. Constant temperature has proved to be unnecessary. The distillation step separates the arsenic from silica and phosphorus, which also give blue-colored molybdenum compounds, while other volatile chlorides of antimony, selenium, and germanium do not interfere in the subsequent color development.

It is essential to run a reagent blank for each lot of reagents used, because of their variability in arsenic content (Tables II and III). Dust and other particulate matter such as cigaret ashes contain small amounts of arsenic; therefore it is necesary to guard carefully against dust contamination, particularly when dealing with small quantities of arsenic.

The use of battery-type equipment with adjustable electrical heating facilities makes possible the uniform, rapid, and convenient handling of a large number of samples daily in a small working space. What is more, after the digestion process has started, it requires little attention. The use of interchangeable joints at strategic places in the distillation apparatus also makes the operation more convenient, particularly in respect to rapid cleaning. The accuracy of recovery by this method is superior to that of the method described by Chaney and Magnuson (*1*), and equal to that obtained by Morris and Calvery (*6*), but in the author's hands, at least, the procedure seems to be more rapid and the substitution of the distillation step for the arsine evolution and combustion technique makes for simplicity as well as compactness of apparatus. A further advantage lies in the fact that a single pure color is employed, instead of the "mixed color" obtained by Chaney and Magnuson (*1*).

The method has been found applicable to a wide variety of biological materials and has been especially useful in the analysis of the normal daily fecal and urinary excretions of rabbits, which entails an accurate determination of arsenic in amounts of less than 10 micrograms, and in the analysis of small samples of blood and spinal fluid. Several modifications would aid in accelerating the analyses in certain cases—for example, when extremely large amounts of arsenic are present, the distillate may be titrated directly by Scherrer's method (*8*). Again, the possibility of determining arsenic polarographically is being investigated in this laboratory, and the preliminary work promises a sensitivity comparable to that of the method here reported, with the advantage of increased simplicity. It is hoped that the data on this development will be available shortly.

TABLE III. RECOVERY OF KNOWN AMOUNTS OF ARSENIC ADDED TO WHOLE BLOOD

(5-gram samples)

Range Used *Micrograms*	Arsenic Added *Micrograms*	Arsenic Found *Micrograms*	Arsenic Recovered *Micrograms*
0– 10	Nil	0.5[a]	...
0– 10	Nil	0.7[a]	...
0– 10	Nil	0.6	...
0– 10	Nil	0.7	...
0– 10	1.0	1.7	1.1
0– 10	1.0	1.5	0.9
0– 10	5.0	5.5	4.9
0– 10	5.0	5.7	5.1
0– 50	15.0	16.0	15.5
0– 50	15.0	15.5	15.0
0– 50	50.0	50.5	50.0
0– 50	50.0	50.0	49.5
0–100	100.0	100	100
0–100	100.0	99	99

[a] Calculated as reagent blank, 5 grams of distilled water being substituted for 5 grams of whole blood.

Summary

A photometric method has been applied to the microdetermination of arsenic in small samples of biological material—for example, 100 ml. or less of urine and 10 grams or less of blood or other tissue.

The sample is prepared for analysis by the wet-ashing method, employing perchloric, nitric, and sulfuric acids. The arsenic contained in the ashed sample is isolated completely

from other interfering constituents, such as silica and phosphorus, by distillation as the trichloride, in a distillation apparatus designed to ensure ease of cleaning and charging. The arsenic is oxidized to arsenic acid and the density of the blue color obtained by formation of the stable arsenic-molybdenum compound is measured photometrically.

Three ranges are used for the quantitative estimation of the arsenic—namely, 0 to 10, 0 to 50, and 0 to 100 micrograms—the accuracies for the above ranges being, respectively, ±0.1, ±0.5, and ±1.0 micrograms. A reagent blank which varies from 0.5 to 2.0 micrograms is run simultaneously with each set of analyses and it is determined with an accuracy of ±0.1 microgram, thus securing an accuracy of ±0.2 microgram for the lowest range.

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Apparatus for Extraction of Lipoids from Wet Tissues

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THE small apparatus shown in Figure 1 has been used in this laboratory since 1932 for the extraction of lipoids from blood in the determination of cholesterol. The method used has been published only in the abstract (*8*) without a sketch of the apparatus. The essential modification in this extractor from previous ones of similar type, such as those of Leiboff (*12*, *13*), Ling (*14*), and numerous others, is the trap fixed to the reflux condenser at its lower end. Moisture from the sample distills with the solvent and is retained in the trap. A notable improvement in consistency of results has been obtained, and there are reasons for concluding that direct extraction with chloroform under these conditions results in complete, or nearly complete, removal of cholesterol from the sample. However, although most of the author's work has been done with chloroform as the solvent, this extractor, and the larger ones shown in Figures 2 and 3, are adaptable to use with other solvents.

Microextractor

The apparatus (Figure 1) consists of an extraction tube having a bulb at the bottom calibrated to contain 5 ml. of extract and four lugs to support the sample, and a separate one-piece assembly of condenser and trap which fits into the top part of the tube. The body of the tube above the constriction is of 24- to 25-mm. tubing and has an approximate length of 230 mm. The four lugs, shown laterally and in section, are approximately 50 mm. above the top of the constriction. The constricted part is of 11-mm. tubing and has a length of 13 to 15 mm., these dimensions having been found best to give sufficient accuracy in making up to volume and still to permit rapid and thorough mixing of the extract with the reagents in the determination of cholesterol. The condenser has a long skirt which serves to hold it and the trap in the center of the extraction tube and also to deflect any moisture from the air down the outside of the apparatus. The extraction tubes and condenser skirts are kept within close enough tolerances so that a large number of tubes and several condensers are interchangeable. The outer shell of the trap and the part of the condenser hanging in the extraction tube are of 16-mm. tubing. The inner tube of the trap is of 11-mm. tubing extending at least 12 mm. below the ring seal joining it to the shell, and the shell extends like a collar at least 12 mm. above the seal. The openings through the supporting member connecting the trap to the condenser have a diameter of not less than 5 mm. The holes to the outside through the shell below the ring seal have approximately 3 mm. diameter. The bottom of the trap should be between 15 and 30 mm. above the lugs.

For operation, 5 to 6 ml. of solvent are placed in the bulb, a piece of fat-free filter paper 20 × 20 × 1 mm. with its corners bent at right angles is dropped "ears up" onto the lugs, 0.25 ml. of the specimen (serum, heparin plasma, whole blood, "saline" suspension of cells, or other fluid) is delivered onto the paper from a graduated 1-ml. pipet or from a special pipet (*7*), and the extraction tube with the condenser in place is immersed to the level of the sample in a boiling water bath. (A tall-form 400-ml. beaker makes a satisfactory bath for two tubes.) The extraction is continued for 1 hour.

Vapor of the boiling solvent passes around and bathes the sample, carrying moisture from the sample to the condenser. The condensate flows into the trap through the openings in the supporting member. Solvent and water separate in the inner tube of the trap, the water being retained and the solvent flowing around the bottom of the inner tube

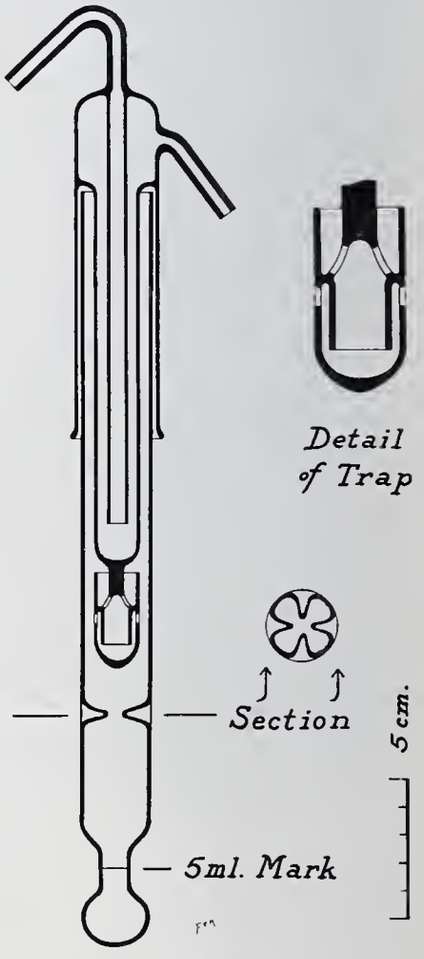

Figure 1. Microextractor for Extraction of Lipoids from Blood
Condenser and trap in position in tube

and out the small holes in the outer shell of the trap and dripping back onto the sample (heavy solvents).

For solvents lighter than water, a simple cup supported by a rod extending to the bottom of the cup seems to be adequate. Such a trap has been tested with ethyl ether in two or three determinations.

A trap suitable for both heavier and lighter solvents could be made by extending the outer shell of the trap shown in Figure 1 about 15 mm. below the bottom of the inner tube. Such a trap has not been tested.

Large Extractors with Water Traps

The performance of the microextractor (discussed below) suggested the use of similar apparatus for extractions on a larger scale.

In 1940, Kaye and co-workers (*9*) described an apparatus for drying and extracting specimens in a practically continuous operation without transfer from the one apparatus. The water trap in Kaye's apparatus could easily have been redesigned to retain water above a heavier solvent. Instead, the entire apparatus was redesigned to accomplish several objectives: (1) to eliminate the large ground joints at the top of the extraction chamber and below the reflux condenser, (2) to eliminate fragile outside tubes, and (3) to make the parts of the apparatus coaxial in order to save space and to eliminate the extra water bath. Whether or not these objectives are also advantages depends upon the personal preference of the person using the apparatus.

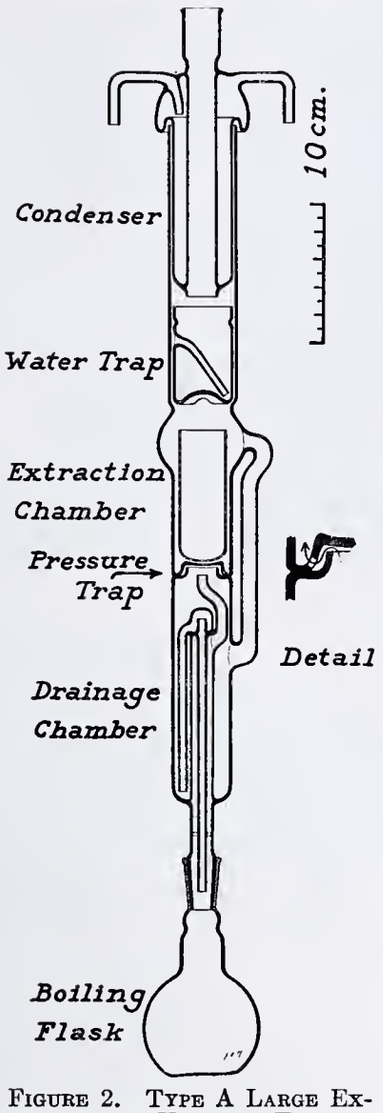

FIGURE 2. TYPE A LARGE EXTRACTOR FOR USE WITH THIMBLE

Left half of pressure trap in detail at right; arrow shows path of vapor

The first extractor tested is shown in Figure 2. This was again redesigned with the object of forcing vapor through the sample to produce vigorous agitation of the sample in the solvent, and the extractor shown in Figure 3 was built.

These two extractors have been tested only to the point of performing extractions in them. It was intended to compare extracts of wet material with those of dried material in respect to total lipoid extracted, volatile constituents, and possibly the changes in labile constituents such as unsaturated fatty acids, vitamins, etc., but this work has been indefinitely postponed. It seems advisable to make the apparatus available now, rather than to wait until work can be resumed.

LARGE EXTRACTOR FOR USE WITH A THIMBLE. In the extractor shown in Figure 2, Type A, solvent vapor from the boiling flask passes upward through the large center tube (containing the siphon) into the drainage chamber, through the vapor tube (side tube, not labeled) to the extraction chamber, and upward to the condenser. The reflux fills the water trap and flows down the walls of the extraction chamber and back into the drainage chamber. When the drainage chamber becomes full enough to cover the lower end of the vapor tube, solvent is forced up into this tube, and the pressure of the vapor is increased, forcing it through the small amount of reflux collected in the pressure trap. The purpose of the pressure trap is to prevent drainage of all but a small portion of the liquid solvent from the extraction chamber. Small increments of refluxed solvent draining into the lower chamber raise the level in the vapor tube and hence the pressure of the vapor below the trap, so that the vapor can support, and is forced through, an increasing depth of liquid in the extraction chamber.

When solvent in the extraction chamber reaches sufficient depth to fill the thimble to within about 8 mm. of the top edge, it begins to splash over into the vapor tube. The bulblike expansion in the top of the extraction chamber allows this splash to be directed outward toward the wall instead of into the top of the thimble. Continued increase of solvent in the extraction chamber results in overflow into the vapor tube, and this excess flows into the drainage chamber until it overflows through the siphon. Drainage of solvent from the thimble can then continue during a considerable period of time until the level of liquid in the lower chamber again reaches the bottom of the vapor tube.

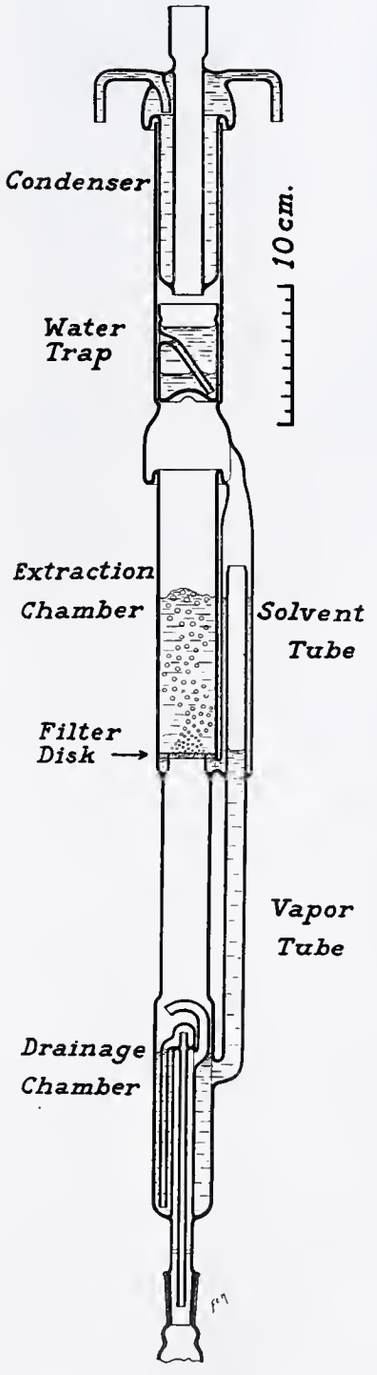

FIGURE 3. TYPE B LARGE EXTRACTOR PRODUCING AGITATION OF SAMPLE

The pressure of the vapor against the liquid in the drainage chamber is practically equal to that exerted through the siphon from the boiling flask, and consequently the siphon operates like an ordinary siphon at atmospheric pressure. It is essential that the top of the siphon be correctly designed to prevent dripping and failure to trip. The design shown here has worked well. The arm of the siphon in the drainage chamber should have a slightly larger inside diameter than that of the arm descending into the boiling flask (inside the large center tube), and should be of a size that will not entirely damp the small surges of liquid which aid in tripping the siphon. Inside diameters of 4 and 3 mm., respectively, were found to be satisfactory. To prevent its tripping too soon, the top of the descending limb of the siphon should be between 15 and 20 mm. above the level of the top of the opening into the vapor tube.

The extractor may be built in two parts, the upper diaphragm of the trap being formed on the lower end of the extraction chamber and the lower half of the trap on the top of the drainage chamber. The upper and lower halves of the vapor tube may then be joined, and while the glass is still soft, the halves of the

trap may be brought together and fused, a fine glass rod being used to encircle the junction, filling any cracks and forming a smooth ring seal. Immediate thorough annealing is necessary.

LARGE EXTRACTOR PRODUCING AGITATION OF SAMPLE. In the extractor shown in Figure 3, Type B, the operation of the siphon and solvent vapor ducts is the same as in the Type A apparatus. The solvent backs up in the vapor tube and produces pressure on the vapor in the chamber below the filter disk in the same manner. However, the reflux, instead of flowing down the walls of the extraction chamber, is caught in the annular channel at the top of the chamber and directed down the solvent tube. It passes down this tube, up through the outer portion of the filter, over the filter, and down through the center part of the disk (Figure 4), flushing any residual extract from the preceding cycle out of the bottom of the mass being extracted. When the pressure of the vapor below the disk becomes sufficiently great to stop this flow, bubbles of vapor begin to pass upward into the extraction chamber. As soon as the hot vapor has warmed the solvent in the extraction chamber to its boiling point, the bubbles pass through the chamber, producing an almost violent agitation of the solvent and of the solid material suspended in it.

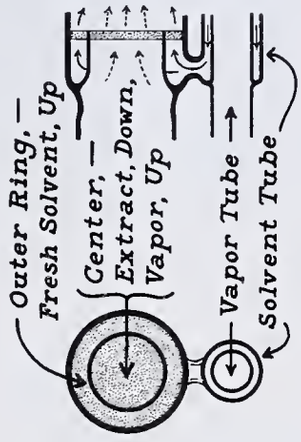

FIGURE 4. DETAIL OF FILTER DISK IN TYPE B EXTRACTOR

Plan below and longitudinal section of column above

The pressure required to force liquid solvent up through the outer part of the filter is so low that the level of the top of the liquid in the solvent tube remains within a few millimeters of that of the liquid in the extraction chamber. When the liquid in the extraction chamber has been sufficiently augmented by reflux from the condenser and water trap, solvent flows over the top of the vapor tube and down into the drainage chamber, raising the level in this chamber gradually until it trips the siphon. The drop in pressure resulting from the emptying of the vapor tube permits solvent to drain from the extraction chamber through the filter. After flow stops in the siphon, all the solvent in the extraction chamber can be accommodated in the drainage chamber. Extract and the continuing reflux fill the chamber to the bottom of the vapor tube, and the cycle of operations begins to repeat itself.

The relationship between the height of column in the vapor tube and the porosity of the filter disk in the extractor tested is satisfactory for a disk of the porosity of the one described. The disk used was rather coarse. A greater head, to force vapor through a finer filter, could be obtained by making the vapor tube and drainage chamber longer. In the apparatus used, a maximum elevation of solvent (chloroform) of 370 mm. in the vapor tube could be reached, but it actually operated most of the time at between 230 and 300 mm.

The filter disk was made by sintering powdered Pyrex in a 50-ml. beaker, with the center tube in place which divides the filter into central and peripheral parts. A layer 1 mm. deep of 60- to 80-mesh grains was spread on the bottom of the beaker and over it was laid a layer 2 mm. deep of 40- to 60-mesh grains. The center tube, cut to final length, was pushed into the ground Pyrex to the floor of the beaker, and the beaker and contents were heated in a furnace at about 800° C. until the walls of the beaker began to thicken and bend slightly. After annealing, the glass of the beaker was ground away against a grindstone (Norton, No. 37150-1).

Water Traps and Condensers

The trap of the microextractor is not suitable for measuring the volume of water collected, and separation of trap and condenser would sacrifice the convenience and rapidity with which they can be handled. The separate construction of trap and condenser in the large extractors facilitates interchange of parts and removal of the water collected. The condenser shown has been adequate.

A variety of traps could be designed for the large extractors. Of several types tested, that shown, for solvents heavier than water, retains the least amount of moisture when its contents are poured into a cylinder or buret for measurement. The Type B extractor would accommodate a deeper trap having an inner tube, similar to the one in the trap of the microextractor, graduated for direct reading of small amounts of moisture. However, from large samples having the water content of fresh tissues, the amount of water recovered is so large that the loss of one or two drops would constitute a small error. For the micro- and Type A extractors, the method of weighing the dry residue is always available. Traps for solvents lighter than water were not tested. A plain cup similar to the trap shown but without the overflow tube is suggested. A small powder funnel in the top would direct water below the surface. Small amounts of moisture can be recovered with a pipet with capillary tip, and can be measured by drawing up chloroform below the water until the water is in the graduated part.

A satisfactory tool for inserting or removing the trap or extraction thimble can be made of 3-mm. (0.125-inch) drill rod or 4-mm. (0.156-inch) brass rod. A deep V with a short coil at the apex is made by bending the rod one and a half times around a piece of pipe. The ends are then bent outward slightly to engage the top of the trap or the inside surface of the thimble.

Performance of Microextractor

Since the apparatus is adaptable for use with other solvents and for the extraction of other lipoids, its performance in the extraction and determination of cholesterol is a rather narrow criterion of its general usefulness. However, the results shown in Table I are satisfactory in significance and implication as to the possibility of approaching complete extraction, especially when it is considered that, up to 6 or 8 per cent, a large part and probably the greater part of the differences can be attributed to small variations in both the rate of development and the reading of the Liebermann-Burchard color. The consistency of values of duplicates, in the first line of the table, suggests a close approach to a limit.

The comparison of extraction in the microextractor and colorimetric determination of cholesterol with alcohol-ether extraction and determination by the digitonide method of Kirk, Page, and Van Slyke (*11*) must be interpreted with caution. Excess color due to esters (Kelsey, *10*) could be thought to have compensated for and concealed a deficiency in the extraction, particularly a failure to extract a definite portion associated with the proteins (*5*, *6*). That the deficiency, if any, in the extraction itself is much smaller than might be inferred, is shown by the small amount of residual cholesterol found after saponification in previously extracted samples (saponified, acidified, and re-extracted). The explanation of the small effect of the esters and of other substances producing excess color (*2*, *4*, *15*, *16*) has been learned only in part. "Recovery" of added cholesterol, since it is so easily extracted, has only the negative value of showing that no gross errors occurred in the colorimetry.

TABLE I. PERFORMANCE OF MICROEXTRACTOR

Nature of Data[a]	Number of Trials	Differences Found Maximum %	In 95% of cases %	Average %
Difference between duplicate colorimetric determinations on chloroform extracts	603	13	<6.2	0.7
Comparison of values in same sample by above method and by Kirk's method	65	15	<±9.2	+1.3
Recovery of added cholesterol by above method (per cent of total, that in sample + that added)	9	−4.3	<±4.1	−1.0
Re-extraction after saponification of extracted sample (24 samples combined, yield = 22 mg. %, Wrattan No. 71-A red filter in eyepiece, extract, brown)	(Color, olive green)			<5.3

[a] First-named method against second as standard.

Table II. Colorimetric Values

Nature of Data[a]	Number of Trials	Differences Found: Maximum %	In 95% of cases %	Average %
Comparison of readings on same extracts through red filter with direct readings of green color after extraction in microextractor	63	− 7.4	<− 4.2	1.0
Similar comparison after alcohol-ether extraction and careful drying in open beaker	11	−35	<−20	−15
Comparison on same specimens of color from alcohol-ether extract with that from microextractor	11	+23	<±18	+11

[a] First-named method against second as standard.

Use of Chloroform as Solvent

Chloroform was used in the large extractors merely as a starting point, because it was convenient and had given good results in the small apparatus. A. C. P. grade containing "up to 1 per cent" absolute alcohol was used. There is no purpose implied here of an indiscriminate defense of its general use as a lipoid solvent.

It is generally accepted that alcohol-ether extraction of blood, serum, etc., is complete. However, Mueller (*16*) and others have found, and the author's data confirm the fact, that such extracts contain substances which produce excess of color in the Liebermann-Burchard reaction if the alcohol-ether extract is taken to dryness in an open beaker, even with the utmost care. Bloor (*3*) avoids this difficulty by evaporating off the solvent in a flask covered by a watch glass, and others interpose an additional step in which the residue is extracted with petroleum ether. Chloroform extraction in the microextractor solved the difficulty in this laboratory. Not only is it not necessary to evaporate to dryness, but if an extract accidentally goes dry, the residue appears to be much less sensitive to these color-producing changes. A comparison of colorimetric values obtained after chloroform extraction in the microextractor with those found after careful evaporation of alcohol-ether extracts in open beakers is given in Table II. The light filter (Wrattan No. 71-A), transmitting above 620 mμ, decreases or eliminates the effect of yellowish tints on the readings. Since esters are equally present in both kinds of extract, it is probable that other substances are involved in the discrepancy.

Performance of Large Extractors

Type A. The operation of the extractor was reliable and satisfactory except as noted. Considerable latitude was permissible in the rate of refluxing and boiling of the solvent. Chloroform was used. The rate of drying of the specimen was disappointing; about three times as much time was required to drive off moisture as with the Type B extractor. This extractor would be useful when ample time could be allowed, and under such circumstances its fully automatic operation would effect a saving in actual working time.

Type B. A mash of canned peas containing 83 per cent of moisture required about 20 hours to dry to constant weight in an oven at 90° C. No significant amount of weight was lost on further drying at 105° C. From 62 grams of the original wet mash in the chamber of the extractor shown in Figure 3, water equal to 77 per cent was recovered in the trap in 12 hours, using chloroform as the solvent. At this time the extraction seemed to become accelerated as gaged by the increase of green color in the extract. After 8 hours more, 83 per cent had been recovered as water in the trap. These rough measurements serve sufficiently well as a basis for comparison. The drying and extraction appear to be somewhat faster than the conventional drying and extraction of the same material separately.

It was planned to use the apparatus for the extraction of animal tissues, chopped, rather than ground, to avoid loss of watery constituents. The conditions of the extraction were, therefore, made intentionally more than ordinarily rigorous. Pieces of the seeds ranging from 2 to 5 mm. across and large pieces of seed coat were seen in the extracted residue.

Discussion

It is hardly necessary to present data or cite references in support of the assertion that the extraction of wet samples by chloroform in extractors of the Soxhlet or Leiboff types is incomplete. Extraction by chloroform or ethyl ether from carefully dried samples may be nearly complete or far from complete, depending upon circumstances. Preliminary experiments in this laboratory showed three out of seven extractions of air-dried samples to lack more than 10 per cent of completion in apparatus identical with the microextractor except that it had no trap. The water trap definitely contributed to an improvement in the chloroform extraction. Since alcohol dissolves small amounts of nonlipoid material, and since acid or alkaline hydrolysis may produce undesired changes, any improvement in extraction may be of definite value.

A few experiments, using other alkyl chlorides in the microextractor, tend to show that the chemical nature of the solvent, and not the temperature of the extraction, determines the completeness of extraction (*5*) in one hour's time.

It may be of some importance in the rate of drying the sample that the water vapor appears to be layered above the chloroform vapor, but this observation was not subjected to comparative test.

An important advantage of conducting the drying and the extraction in the same vessel, in the presence of the solvent and with the outlet closed by the condenser, was pointed out by Kaye and associates—volatile fractions were retained that would be lost by drying with heat in an open vessel.

Agitation in the Type B apparatus undoubtedly contributes much to the rate of both drying and extraction; there is no chance for imprisonment of moisture in lumps of particles of the sample, all surfaces of every particle are continuously bathed in moving solvent, and the temperature of the solvent is maintained at near its boiling point throughout the chamber.

It is likely that minute coagula or varnishlike films may oppose a barrier to the extraction of portions of material (*1*). The writer was unable to demonstrate such coagula or films microscopically, but they might well be of submicroscopic size. However, the pea mash dried in the oven showed a shiny varnishlike appearance and adherence of particles in mosslike masses, while that dried in the solvent in the extractor (Type B) below a water trap showed discrete particles down to the fineness of a powder with "flat" powdery-appearing surfaces.

As for the mechanism of the extraction, it is conceivable that, with solvent present as the material dries out, the solvent enters the material in more minute division and in more intimate contact with its ultimate particles. In a hypothetical picture the solvent enters interspaces before they are closed by the receding of the moisture. The marked tendency of chloroform to form emulsions with water may be a factor, to which the trace of alcohol may contribute. Since the material goes through all degrees of moisture content, it must at some point pass through the optimum for extraction.

Summary

Three extractors are described in which the moisture of the specimen or material to be extracted is driven off in the presence of the solvent and its vapor. In the micromethod, at least, specimens are extracted more nearly completely than when they have been previously dried or when they remain wet throughout the extraction. The moisture is removed in all three extractors by means of a trap interposed in the reflux between the condenser and the sample. One extractor provides for vigorous agitation by a stream of bubbles of solvent vapor flowing upward through the solvent and sample in the extraction chamber. In all three, the specimen is dried and extracted in the same apparatus without interruption of its automatic operation. The parts are coaxial to save space.

Acknowledgment

Nearly all the determinations by the method of Kirk, Page, and Van Slyke were made by Mildred Kaucher.

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Estimation of Small Amounts of Antimony with Rhodamine B

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Rhodamine B reacts with Sb^V in hydrochloric acid solution or sulfuric acid solution containing chloride ion to form a red, water-insoluble stable compound, soluble in organic solvents. The excess reagent is destroyed with bromine, the rhodamine B complex put into solution by addition of alcohol, and the amount present estimated colorimetrically. Practically all acid radicals except chloride and sulfate interfere, as do arsenic, iron, and certain metals of the dithizone group. The latter interference can be avoided by preliminary extraction with dithizone. The method will detect as little as 0.1 microgram of Sb^V in 5 ml. of solution, and is rapid, inexpensive, and specific. The exact procedure is described for the concentration range 0.1 to 300 micrograms of antimony.

THE determination of small amounts of antimony, such as are encountered in tissue and air hygiene analysis, is extremely difficult and in some instances impossible with the usual methods for determining this metal. The chemistry of antimony and its compounds has been inadequately investigated and frequent cases of anomalous behavior are encountered in ordinary analytical experience. The difficulty becomes more marked when the concentration of the metal in the sample under study falls below 1 mg. The industrial hygienist and toxicologist have need for a method which will function in the range 1 to 1000 micrograms.

This paper reports the result of an investigation of the reaction between Sb^V and rhodamine B and describes a colorimetric procedure based upon this reaction.

Reagents

Sulfuric acid, 25 per cent solution, 125 ml. of sulfuric acid, specific gravity 1.84, made up to 500 ml. with distilled water.

Ceric sulfate, 0.1 *N* solution, 3.3 grams of anhydrous ceric sulfate made up to 100 ml. with 3 per cent sulfuric acid.

Sodium thiosulfate, 0.1 *N* solution, 2.5 grams of sodium thiosulfate pentahydrate made up to 100 ml. with distilled water.

Lithium chloride solution, 25.5 grams of lithium chloride made up to 100 ml. with distilled water.

Saturated bromine solution, an excess of liquid bromine in approximately 100 ml. of distilled water.

Rhodamine B, 0.2 per cent solution, 1 gram of rhodamine B dissolved in 500 ml. of distilled water and filtered. The rhodamine B used in this work was a practical grade obtained from the Eastman Kodak Company.

Hydroxylamine hydrochloride, 1 per cent solution, 1 gram of hydroxylamine hydrochloride made up to 100 ml. with distilled water.

Ethanol, 95 per cent, or absolute methanol.

Standard Solutions

Dissolve 0.5 gram of reagent grade antimony metal in 125 ml. of hot concentrated sulfuric acid, cool, and dilute to 500 ml. Dilute 50 ml. of this solution to 500 ml. with 25 per cent sulfuric acid solution; 1 ml. of the dilute standard contains 100 micrograms of antimony. Experience in this laboratory indicates that a solution of the theoretical amount of undried tartar emetic will serve as a satisfactory standard. Samples obtained from three manufacturers (Mallinckrodt, Merck, and J. T. Baker) were found to be identical in antimony content. A given lot of tartar emetic should be checked against an antimony metal standard before use.

To prepare a standard for color comparison, add an appropriate amount of the dilute standard solution from a 5- or 10-ml. microburet to a 50-ml. glass-stoppered graduated cylinder and make the total volume up to 5 ml. with 25 per cent sulfuric acid, using this solution to rinse down the sides of the cylinder. Then develop the color as described below. Standards should be made in duplicate and for colorimeter work should be within 25 micrograms of the unknown for best accuracy. The color is stable and the standard may be kept for several days without showing color change.

Procedure for Colorimeter

At the start of the analysis, the antimony must be in an approximately 25 per cent sulfuric acid solution. Place from 1 to 5 ml.

of the sample (keeping the total antimony content between 50 and 250 micrograms) in a 50-ml. glass-stoppered graduated cylinder. Bring the total volume to 5 ml. with 25 per cent sulfuric acid solution, adding the solution down the sides of the cylinder by means of a buret. Add 1 ml. of lithium chloride solution, mix, add 0.1 *N* ceric sulfate solution (approximately 2 drops) until a pale yellow color persists, and then add sufficient 1 per cent hydroxylamine solution (approximately 2 drops) to discharge the yellow color. Cool in an ice bath for 15 minutes. Remove, add 1 ml. of 0.2 per cent rhodamine B solution from a 25-ml. buret, mix, and cool in an ice bath for 10 minutes. Add 10 ml. of water and cool in the ice bath for 10 minutes. Remove, add 2 drops of saturated bromine water, quickly mix, immediately add a similar volume of 0.1 *N* sodium thiosulfate solution, and mix. Then immediately add 20 ml. of alcohol, shake, bring the volume to 50 ml. with distilled water, and shake. Let stand 20 to 30 minutes and compare with similar standards in a Duboscq colorimeter. Add all reagents directly to the solution and not down the side of the cylinder. Mixing implies gentle swirling in contrast to shaking. Run blanks on the reagents.

When the antimony content of the sample taken for analysis is below 25 micrograms, use only 0.5 ml. of rhodamine B and make the comparisons in Nessler tubes using the standard series procedure.

Table I. Reagent Required

Antimony Content of Sample *Micrograms*	Rhodamine B Reagent Required *Ml.*
0–50	0.5
50–150	1.0
150–200	1.5
200–300	2.0

Precautions

The bleaching operation is the most critical step in the procedure. The temperature of the solution must be kept below 10° C. to ensure complete insolubility of the complex. Exposure to bromine must be no longer than necessary to produce a bleach. Only a moderate excess of reagent is permissible, and this excess must be promptly destroyed with thiosulfate. If the acidity is too high or too low the bleach is not satisfactory. As bromide ion forms a colored complex with the reagent, the presence of too large an excess of reagent in the sample will give a positive blank. Blank reactions conducted with 1 ml. of rhodamine B reagent should result in no more than a pale pink color and with 0.5 ml. of reagent should be water-white. The proper amount of reagent to use in respect to the antimony content of the sample is shown in Table I.

The colorimetric method described is sensitive to the manipulative procedure used. Most analysts become proficient with the method after 2 or 3 days of practice. Samples containing no antimony should be run until satisfactory blanks can be obtained, then samples containing 100 micrograms of antimony should be run until reproducible results can be obtained. In this laboratory about 4 per cent of the analyses made by this procedure fail for one reason or another. Accordingly, samples should always be analyzed in duplicate. All precautions common to trace analysis, such as scrupulous cleanliness, tested reagents, use of Pyrex glassware, and accurate volumetric glassware, must be observed.

In order to obtain a high sensitivity the volume of solution at the time of reaction must be kept as small as possible. Sensitivity could probably be increased by reducing the final working volume to 10 ml. instead of 50 ml.

Although care has been taken to select quantities of reagents in such amounts as to make their measurement noncritical, reasonable accuracy must be employed in their measurement. The depth of color is dependent upon the alcohol concentration up to about 36 per cent. The sulfuric acid content must be within five per cent of the indicated amount. The volume of water added prior to bleaching should be held to within 2 ml. of the indicated value.

As the color intensity is influenced by temperature, standards and samples should be at the same temperature when compared. The apparent color may be influenced by the deposition of air bubbles on the bottom of the colorimeter plungers; alcoholic solutions are especially troublesome in this respect.

If too large an excess of sodium thiosulfate is added, a turbidity forms, due probably to colloidal sulfur, which interferes with the determination.

Discussion of Method

In a qualitative test utilizing the reaction mentioned in this paper (*1*, *2*), the rhodamine B solution is treated on a spot plate with the test solution strongly acidified with hydrochloric acid and previously oxidized with sodium nitrite. In the presence of antimony, the light red dye solution changes to violet. A quantitative reaction could not be developed from this procedure, because of interference of excess sodium nitrite and excess reagent in the solution at the completion of the test.

Sb^{V} reacts with an aqueous solution of rhodamine B in a solution strongly acid with hydrochloric acid or one strongly acid with sulfuric acid and containing chloride ion, to form a dark red compound, extremely insoluble in aqueous solvents. In fine suspension it has a bluish purple fluorescence and when precipitated in the presence of a protective colloid such as gum ghatti, the suspension appears blue in a colorimeter. Under proper conditions the reaction appears to be quantitative and upon filtering no antimony can be detected in the filtrate. The compound formed is soluble in many organic solvents, filters readily, and is unusually stable. Attempts at analysis have not met with success, but apparently one atom of antimony is associated with one molecule of rhodamine, possibly as a complex salt. The presence of adequate chloride ion is absolutely essential to the reaction, and chloride is present in considerable amount in the final compound. Although several attempts were made to determine the chloride content, using the Parr bomb and the Willard-Thompson method, consistent results were not obtained, perhaps because of variable composition of the compound or failure to obtain satisfactory decomposition of the complex, which is exceedingly stable. It is most conveniently decomposed a few tenths of a gram at a time by digesting with hot 30 per cent fuming sulfuric acid until well in solution and then cautiously adding 30 per cent hydrogen peroxide until the mixture is colorless.

The compound may also be formed in 50 per cent hydrochloric acid solution but a 25 per cent sulfuric acid solution is preferable for colorimetric determination.

The choice of oxidizing agents for converting Sb^{III} to Sb^{V} is limited. If sodium nitrite is used, the excess nitrite interferes with the reaction and must be destroyed with urea, and under some circumstances the nitrate formed by the reaction interferes. Sodium chlorite is satisfactory and preferable if the reaction is carried out in hydrochloric acid solution and the excess reagent is destroyed with hydroxylamine hydrochloride. The reagent of choice is ceric sulfate, when a sulfuric acid solution is used. It serves as its own indicator and the excess is readily removed with hydroxylamine hydrochloride. A slight excess of ceric or cerous ion influences the final color.

It is important that the complex be formed at high acidities if a quantitative reaction is to be obtained. The acidity is not critical except in the colorimetric method, where the shade of the final color is influenced by the acidity and the range in which bromine will function as a bleach is somewhat limited.

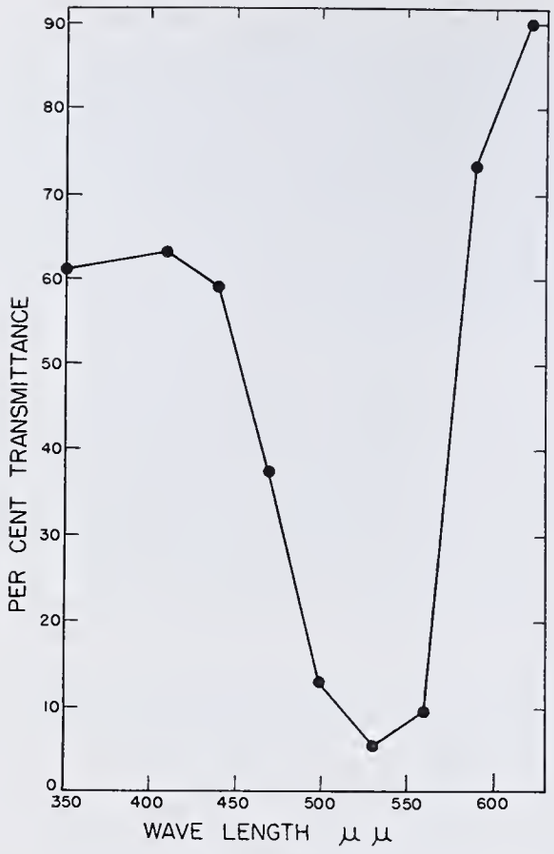

FIGURE 1

Many bleaching agents were tried, but only bromine water is satisfactory. Rhodamine B itself is not easily decolorized. On the other hand, a too active reagent will attack the solid rhodamine B–antimony complex.

The color produced when the complex is dissolved in alcohol appears to follow the Beer-Lambert law in all concentration ranges studied. The ethyl alcohol solution showed a marked absorption to light of about 530 millimicrons wave length as determined by the Coleman R. S. photometer (Figure 1).

Lithium chloride must be used as a source of chloride ion, since it is the only readily available inorganic chloride salt which will not interfere with the reaction and still be sufficiently soluble in 40 per cent alcohol to avoid precipitate formation.

The procedure here described is rapid and requires little unusual or expensive equipment or reagents. In routine, an analyst can conveniently prepare standards and analyze 30 samples in the usual working day if samples are handled in groups of 12 or 24.

Interfering Substances

The effect of several metallic ions, salts, and acids is shown in Table II. Nearly all acid radicals except chloride and sulfate will cause trouble. When the sample contains 10 micrograms or less of antimony, the effect of many interfering substances is greatly diminished. Metals of the dithizone group, such as mercury, copper, nickel, cadmium, and zinc, may be conveniently removed by preliminary extraction of the sample at approximately pH 8.5 with 100 mg. of dithizone per liter of carbon tetrachloride solution, using the usual dithizone technique. Antimony does not appear to react with the dithizone or be lost in the extraction process.

Results of Analysis

Typical analytical results based on synthetic samples of known antimony content are shown in Table III. These data were obtained by five different analysts and represent single determinations on single samples. Some samples have permissible amounts of other compounds present, some represent antimony added to beef tissue and ashed, and some represent results obtained by making the analysis in 50 per cent hydrochloric acid solution, employing sodium chlorite as the oxidizing agent. No attempt has been made to classify the data further, inasmuch as more than 1000 separate analyses of all kinds showed no significant deviation or trends among themselves.

Analysts using this method only occasionally may expect to obtain results within ±5 per cent of the truth for antimony concentrations in the range of 25 to 200 micrograms, and within 25 per cent of the truth for concentrations below 10 micrograms. In routine use somewhat better accuracy may be obtained. Deviations greater than ±10 per cent are rarely encountered in the range of 25 to 300 micrograms of antimony, even though the technique may be faulty. The accuracy and precision have about the same order of magnitude.

TABLE II. INTERFERENCE TABLE

(100 micrograms of Sb^V in sample)

Substance	Amount	Interference Effect	Substance	Amount	Interference Effect
	Mg.			*Mg.*	
H_3PO_4	200.0	None	Ce	1.0[a]	Off color
Al	7.5	None	Hg	0.5[a]	Deepens color
Mn	7.5	None	Cd	3.0[a]	Deepens color
Bi	7.5	None	Zn	3.5[a]	Deepens color
$CaSO_4$	Saturated solution	None	$KClO_4$	Trace[a]	Deepens color
Mg	7.5	None	Sn	3.5[a]	Deepens color
$PbSO_4$	Saturated solution	None	$HClO_4$	Trace[a]	Deepens color
Fe	4.0[a]	Off color	Cu	Trace[a]	Weakens color
$As^{III, V}$	Trace[a]	Off color	Ni	7.5[a]	Weakens color
$NaNO_3$	1.0[a]	Off color	$NaNO_2$	Trace[a]	Weakens color
NaF	1.0[a]	Off color	NaBr	0.5[a]	Weakens color
Tl	Trace[a]	Off color	H_2O_2	Trace[a]	Weakens color

[a] Maximum permissible amounts for samples containing 100 micrograms of antimony.

TABLE III. ANTIMONY RECOVERY

Added	Found	Error	Added	Found	Error
Micrograms			*Micrograms*		
200	195.7	−4.3	50	50.2	+0.2
	199.6	−0.4		50.2	+0.2
	200.5	+0.5		47.5	−2.5
	197.1	−2.9		50.1	+0.1
	198.0	−2.0		49.4	−0.6
	194.3	−5.7		51.2	+1.2
	200.5	+0.5		50.2	+0.2
100	99.0	−1.0	100 (dried beef)	102.8	+2.8
	99.0	−1.0		96.6	−3.4
	98.5	−1.5		98.0	−2.0
	92.2	−7.8		100.5	+0.5
	98.5	−1.5		96.1	−3.9
	101.0	+1.0		101.5	+1.5
	102.6	+2.6		99.6	−0.4

Acknowledgment

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